PRODUCTION OF LIQUID BIOFERTILIZER USING SPENT MUSHROOM SUBSTRATE AND WATERMELON PEELS

ABSTRACT

Biofertilizers are natural fertilizers that contain live biomass. This study demonstrates the production of liquid biofertilizer using spent mushroom substrate (SMS) and watermelon peels using a liquid-state fermentation technique. This is ~~as~~ a result ofcontamination and lossof soil fertility faced by the environment and agricultural farmland as a result of incessant use of inorganic fertilizers that are persistent in the soil. The two waste materials were obtained and placed on a 35-litre drum; 27 litresof water was poured intothe mixture after washing ~~of~~ the watermelon peel and stirred evenly, then allowed to ferment for threeweeks under anaerobic conditions. Filtration of the mixture was done after complete fermentation, and microbiological and physicochemical analysis was carried out on the mixture before and after fermentation. Reduction in total nitrogen, phosphorous, magnesium and potassium concentrationwas observed after fermentation, while iron content increased after fermentation. The following bacterial species were identified using 16S rRNA gene amplicons: *Pseudomonasaeruginosa*, *Stenotrophomonas*, *geniculata*, *Sphingobacteriumdaejeonense* and *Alcaligene faecalis*. A pot experiment was carried out with a planting bag on three setups in duplicates: (i) seed + liquid biofertilizer application, (ii) seed + chemical fertilizer application, and (iii) seed alone. Then, bean seed (Phasolous vulgaris L.) and groundnut seed (Arachis hypogea) were planted in each set up after physicochemical analysis of the planting soil. Growth comparison using germination test was done by measuring the shoot length, leaf length, leave number, leaf width, stem girth and number of branches; it was observed that the treatment with liquid biofertilizer application competed favourably with that of chemical fertilizer treatment and the biofertilizer also serves as a bio-control agent as the leaves of the biofertilizer set up werenot eaten by the caterpillar. In contrast,that of inorganic (chemical fertilizer) was eaten up.

Key words: Liquid Biofertilizer, spent mushroom substrate, watermelon peels, chemical fertilizer, and fermentation.

Introduction

Organic fertilizers that include living biomass or dormant cells of beneficial microbial strains are known as biofertilizers. By populating the rhizosphere and making the nutrient readily accessible and useful to plant root hairs, they may increase nutrient digestion. Bio-fertilizer production is another way of practising agricultural microbiology as it impacts crop growth improvement, soil fertility through organic fertilizer, and plant disease control through antagonism.Soluble phosphate fertilizers are used for crop growth enhancement, and these products or research areas have gained tremendous breakthroughs over the years. Bio-fertilizer production has also been commercialized to increase soil fertility. The use of indigenous microorganisms and fungi for these improvements is better than the introduced strains of microbes. These practices are to impede the harmful use of chemical fertilizers and then reduce the rate of pollution through this natural approach.

The substrate used to grow mushrooms combines various natural materials explicitly prepared for cultivating mushrooms. It is spent because it has already been used in the mushroom house after the harvest yearand disposed.

The spent mushroom substrate is a waste material obtained after oyster mushroom cultivation (*Pleurotus* Spp.). SMS refers to the residual substrates that remain after the crop has been harvested and composted. Mustapha et al.'s (2017) research found that SMS (spent mushroom substrate) retains certain residual nutrients that mushrooms may still use.

However, from an economic standpoint, replacing the substrate and starting a new crop is more cost-effective. SMS is distinguished by its substantial organic matter content, which is beneficial and contains both macro and micro nutrients in ~~both~~ total and accessible forms. Optimal pH, advantageous low C/N ratio, and minimal heavy metal concentration. The predominant focus of research on SMS has been on the enzymatic activity of the natural mycelium and its ability to produce lignocellulose enzymes, including laccase, XY lanase, lignin peroxidase, cellulose, and hemicellulose. This has been done to utilizeSMS as a cost-effective resource for bioremediation, animal feed, and energy feedstock production (Cezary et al., 2021).

Watermelon, scientifically known as *Citrullus lanatrus*, is a fruit of significant economic value. It is considered the second biggest fruit crop in the world, with an output of over 103 million tons worldwide. (FAOSTAT 2018) Approximately 25% of the watermelon peel, which makes up a significant portion of the fruit, is often disposed of or tossed into the environment. This practice poses environmental issues. The transformation of this waste in the environment into valuable chemicals and commodities is restricted owing to a lack of understanding ofpotential conversion methodologies.

The remnants of spent mushroom substrate (SMS) obtained from culture bags after the harvest of mushroom fruiting bodies are referred to as compost or spent mushroom substrate (SMS) (Zhang et al., 2016;). SMS, or spent mushroom substrate, is a readily available biomass residue ~~that is~~ generated in large quantities after crop harvesting in mushroom farms (Moon et al., 2012). Approximately 5 kilograms of secondary metabolite substances (SMS) are generated every kilogram of freshly formed fruiting body. Following the collection of mushrooms, SMS debris is often discarded as refuse, posing a significant difficulty in waste management. In many countries, it is typically composted as a means of disposal (Anjali et al., 2021). The mushroom farming business has grown significantly in recent years, resulting in the yearly production of nearly 5 million tons of solid waste in SMS (Mohd Hanafi et al., 2018).

Materials and method

Study Area

This study was carried out at Choba, Port Harcourt, Rivers State, Nigeria.

Sample collection

The spent mushroom substrate (*Pleurotus ostreatus*) was gotten from mushroom farm in Agric faculty of the University of Port Harcourt whereas the watermelon peel was gotten from the Choba market roadside water melon sellers, they were identified at a herbarium in University of Port Harcourt , Physicochemical analysis of the sample was determined before fermentation process.

Isolation of microorganism

One ml of well agitated mixture of the waste substrate was serially diluted in (10 fold) 9ml of normal saline. For phosphorous solubilizing bacteria, aliquots (0.1ml) of 10^{-3} was spread on pikovskaya media while for Nitrogen fixing bacteria, aliquots (0.1ml) of 10^{-3} was spread on Ashby's mannitol agar and also spreading was done on nutrient agar plate all spreading were in duplicates. The plates were incubated for 24 hours, after district colonies was observed on the plates, the plates were enumerated and then sub cultured into differential media such as MSA, Centrimide agar, mackonkey agar, Ashby's mannitol agar and Pikovskaya media. This was also carried out after the biofertilizer was produced.

Media preparation for nitrogen-fixing bacteria isolation

250ml of Mannitol Ashby agar media was formulated by measuring 5g of Mannitol, 0.05g of Potassium Hydrogen Phosphate (K_2HPO_4), 0.05g of Sodium Chloride (NaCl), 0.05g of Magnesium Sulphate Heptahydrate ($MgSO_4.7H_2O$), 0.025g of Potassium Sulphate (K_2SO_4), 1.25g of Calcium Carbonate ($CaCO_3$) 3.75g Agar-agar then dissolved in 250 ml of Distilled water. After 15 minutes in an autoclave at 121°C, the agar medium was placed onto a petri dish and allowed to harden at room temperature.

Media preparation for phosphate-solubilising bacteria isolation

250ml of Pikovskaya agar media was formulated by measuring 2.5g of Glucose, 1.25g of Calcium Phosphate ($Ca_3(PO_4)$ 0.05g of Sodium Chloride (NaCl), 0.025g of Magnesium Sulphate Heptahydrate ($MgSO_4.7H_2O$), 0.05g of Manganese (II) Sulphate Dihydrate ($MnSO_4.2H_2O$) 0.125g of Ammonium sulphate $(NH_4)_2 SO_4$, 0.125g of Yeast extract, 3.75g of Agar-agar then 250ml of normal saline was poured on the mixture. After 15 minutes in an autoclave at 121°C, the agar medium was placed onto a petri dish and allowed to harden at room temperature.

Identification of Isolates

The pure isolates were identified through their morphological, cultural, biochemical characteristics as stated in a manual of determinative bacteriology according to Bergeys. The biochemical tests that was conducted includes: catalase, oxidase, citrate, indole, TSIA, MR-VP, Sugars (lactose, fructose and sucrose utilization test). Azotobacter sp. were recognized by a clear zone around the colonies on Ashby mannitol agar. Cellular identification of bacterial isolates was carried out using gram staining method.

Biofertilizer Production

The watermelon peel was washed and cut into smaller fragments then weighed using the weighing machine and also, the spent mushroom substrate was weighed and scattered into the drum, both waste materials had equal weight of 5379.00g and 27 litre of distilled water was poured on the mixture and stirred evenly then covered airtight to ensure anaerobic condition, the essence of the high quantity of water was to allow free movement of microorganisms and for fermentation to take place faster (Okoli et al., 2019). The substrate was left for fermentation to take place and on weekly intervals, the mixture was checked to observe if absolute fermentation has taken place. After 3 weeks fermentation period, the mixture was sieved using a sterile sieve into 20 litres gallon and kept under room temperature.

Molecular Identification

DNA Extraction

The ZR fungal/bacterial DNA miniprep kit (manufactured by Zymo Research) was used to extract DNA.

DNA Quantification

Nanodrop spectrophotometry was used to determine the concentration of the DNA and access its purity. Agarose Gel Electrophoresis for DNA and PCR was carried out.

DNA Amplification

Sequencing and Sequence Analysis

The fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD 3-100/1000. The cycles that was conducted were denaturation temperature and times, Annealing temperature and time and Elongation temperature and time. The used Bacterial Primer sequences that were used includes:27F with the sequence 5’

AGAGTTTGATCMTGGCTCAG ‘3 and 907R with the sequence 5’ CCGTCAATTCMTTTRAGTTT ‘3.

Evolutionary Relationships of Taxa
The evolutionary history was deduced using the Neighbor-Joining approach (Saitou et al. 1987). The tree that provides the best or most favorable outcome is shown.

Physicochemical Analysis
Physicochemical analysis of the mixed substrate before fermentation and after fermentation with that of the planting soil was conducted in a pharmaceutical chemistry laboratory, located in University of Port Harcourt where analysis of the nitrogen content, phosphorous, magnesium, iron content, potassium, pH value and temperature value was ascertained.

Collection of Planting Soil Sample
The experimental soil sample was gotten from the university of Port Harcourt Delta campus on a randomized sampling method with the use of trowel and was filtered with a large basket to remove debris such as stones, pieces of plastics etc. then the soil was taken to the laboratory for physicochemical analysis.

Pot Experiment set up
The experimental soil was transferred into garden planting polythene bags of 24.1 length, and 22.2 width, each contained about 2723.25g of soil, then seedlings of beans and groundnut were rinsed with a distilled water and planted on the soil in duplicate set ups. For the biofertilizer application soil, the soil was divided into two and packed in duplicate bags also. The beans, groundnut and experimental soil sample were all identified in the department of crop and soil science, faculty of agriculture, university of Port Harcourt. The seeds of beans and groundnut for soil and foliar application were soaked in 5ml of the liquid biofertilizer and chemical fertilizer according to wang et al (2014). For each crop, three set ups were considered in duplicates which includes: liquid biofertilizer + soil + seed, chemical fertilizer + soil + seed (positive control) and soil + seed only which is serves as the negative control. The seeds were distributed in each set up randomly after been subjected to viability treatment.

Seed Viability Test
The both seed of groundnut (*Araichis hypogea*) and beans (*Phaseolus vulgaris*) were subjected to viability test by spreading up to 30 pieces each of the test crop on a tray slightly covered with soil and covered with a polybag to create humidity (Ibiene et al 2012). After 4-5 days of planting, over 90% of the seeds germinated which is indicative that the seeds are viable.

Application of Chemical Fertilizer
The chemical fertilizer were applied according to manufacturer’s instruction, the seeds of beans and groundnut were firstly rinsed using distilled water and sundried, after which soaked in 1.5ml of the chemical fertilizer for 30 minutes before planting on PE5 and PE6 accordingly, foliar and soil application started 10 days after germination of crops. The application continued within the interval of 7 days according to Okoli et al (2019).

Application of Liquid Biofertilizer

The seeds of beans and groundnut for this application were also firstly rinsed using distilled water and sundried after which they were soaked in 5ml of liquid biofertilizer and 5ml of the liquid biofertilizer was sprayed on the sterilized soil to enhance the microbial activities of the soil. Since our biofertilizer contains live organisms that are capable of bringing about aeration and accessibility to some essential nutrients due to their presence in the soil. The soil application and foliar applications commenced after 10 days of seed germination and continued at an interval of 2 days. This application was done and monitored for 5 weeks.

Growth Parameters measurement

The growth parameters for germination test analysis were measured according to Criollo et al (2011) which included the plant height (cm), the number of leaves, leave length (cm) and the stem girth.

RESULTS and DISCUSSION

Table 1: Biochemical test results for isolation before fermentation (BF)

Sample code	Morphology &colour	Shape	Gram stain	Catalase	oxidase	Citrate	lactose	glucose	Sucrose	Slope	butt	gas	H_2S	MR	VP	Tentative identity
MP1. CENTRI.	pink	rod	-	+	+	+	-	+	-	R	R	-	-	+	-	*Pseudomonas* sp.
SSA.	pink	rod	-	-	-	+	-	+	-	R	R	-	-	-	-	*Bacillus* sp.
MSA.	purple	+	+	+	-	+		+	-	R	R	-	-	+	-	*Staphylococcus* sp.
EMB (2).	pink	rod	-	+	-	+	-	+	+	R	R	-	-	+	-	*Pseudomonas* sp.
EMB(3).	pink	rod	-	+	-	+	-	+	-	R	R	-	-	+	+	*Pseudomona* sp.
EMB1.	Pink	rod	-	-	-	+	+	+	-	R	R	-	-	+	+	*Pseudomonas* sp.
NA1.	pink	rod	-	+	-	+	-	-	+	R	R	-	-	+	-	*Pseudomonas*sp
NA2.	pink	rod	-	-	+	+	-	-	-	R	R	-	-	-	+	*Pseudomonas* sp.
NA 3.	pink	rod	+	+	-	+	+	-	-	R	R	-	-	-	-	*Baccillus*sp
NA 4.	Pink	rod	-	+	-	+	+	-	-	R	R	-	-	+	-	*Pseudomonas* sp.

Table 2 Biochemical characterization of isolates after fermentation (AF)

Sample code	Morphology &colour	Shape	Gram stain	Catalase	oxidase	Citrate	lactose	glucose	Sucrose	Slope	butt	gas	H_2S	MR	VP	Tentative identity
PIV1.	pink	rod	-	+	-	+	+	+	+	R	R	-	-	+	-	*Azotobacter* sp.
PIV2	pink	rod	-	+	+	+	+	+	+	R	R	-	-	-	+	*Pseudomonas* sp.
PIV3.	pink	rod	-	+	+	+	-	+	-	R	R	-	-	-	+	*Clostridium* sp.
AM1	pink	rod	-	+	+	+	-	-	-	R	R	-	-	+	+	*Clostridium* sp.
AM2	pink	rod	-	+	+	+	-	+	-	R	R	-	-	+	+	*Azotobacter* sp.

Bacterial isolation

Table 1 and Table 2 shows the morphological and biochemical characterization of bacterial isolates before fermentation process took place and after fermentation process. The results confirmed the presence of microorganisms in the substrates used to make biofertilizers and the population of microbes in the finished product was narrowed down to anaerobic microorganisms though aerobic microbes were present in the substrate before the fermentation process. This was as a result of the anaerobic condition that was maintained during the fermentation period. The nitrogen fixing species and *Azotobacter* species were recognized with a clear zone around the colonies on the growth medium.

MOLECULAR RESULTS

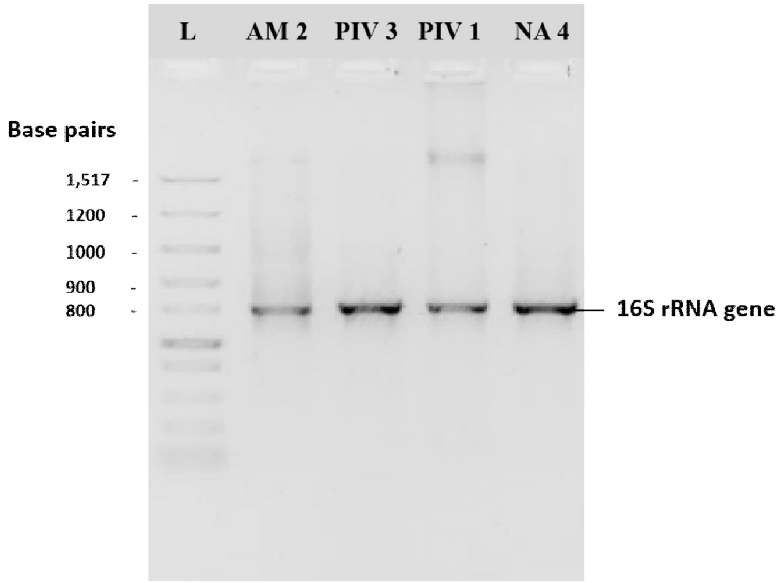

Plate 1: Agarose gel electrophoresis of the 16S rRNA gene amplicons from the bacterial isolates

Table 3: NanoDrop spectrometry characteristics of the DNA from the isolates

S/N	Isolate code	A260	A280	Purity ()	DNA Concentration (ng/µl)
1	AM 2	14.43	7.48	1.93	721.4
2	PIV 3	11.89	5.98	1.99	594.7
3	PIV 1	38.30	18.78	2.04	1914.9
4	NA 4	6.92	3.76	1.84	346

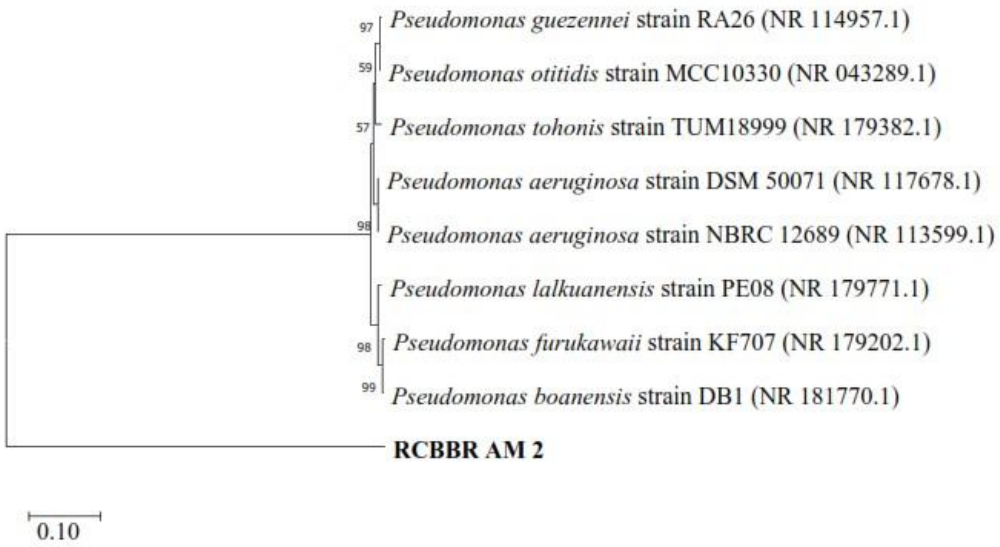

Figure 1: Neighbor-joining evolutionary relation of isolate RCBBR_AM 2 with the GenBank closest matches.

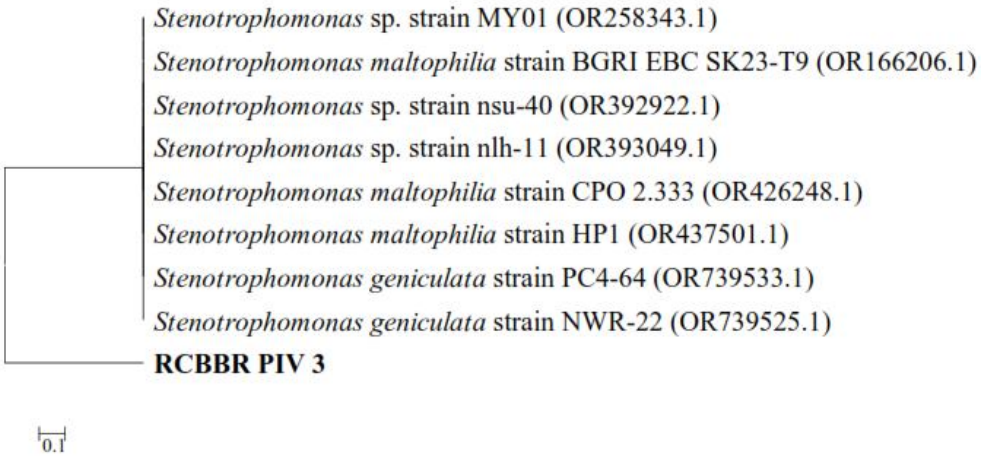

Figure 2: Neighbor-joining evolutionary relation of isolate RCBBR_PIV 3 with the GenBank closest matches.

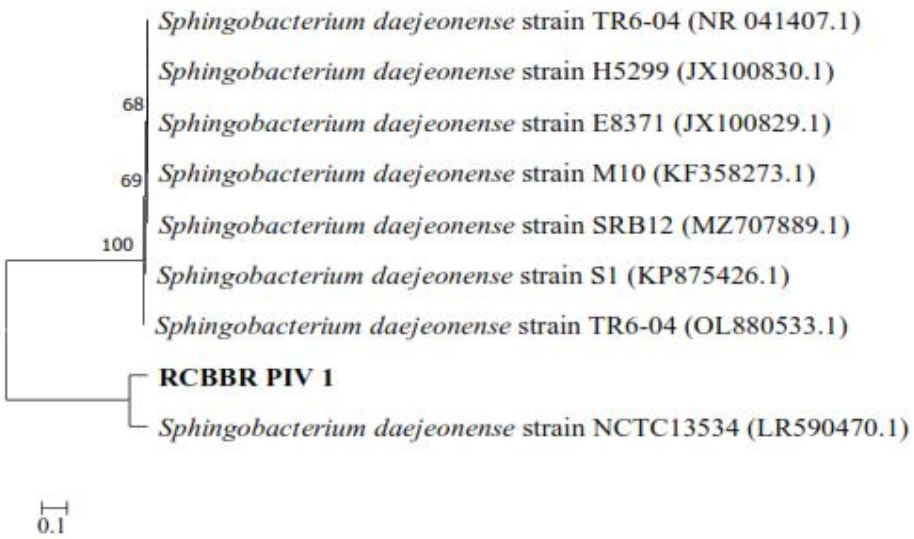

Figure 3: Neighbor-joining evolutionary relation of isolate RCBBR_PIV 1 with the GenBank closest matches.

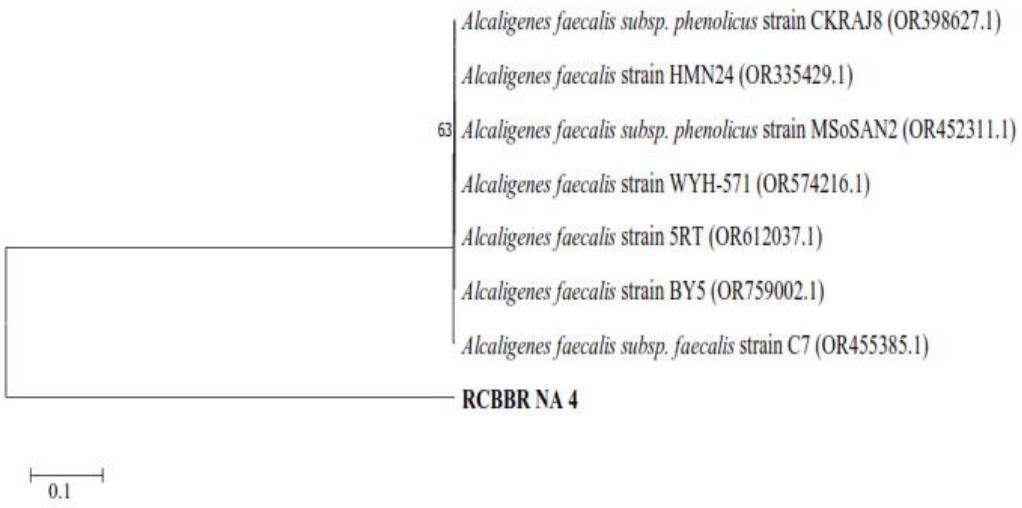

Figure 4: Neighbor-joining evolutionary relation of isolate RCBBR_NA 4 with the GenBank closest matches

Molecular Identificationusing 16S RNA

Table 3 shows the NanoDrop spectrometric characterization of the DNA of the bacterial isolates and it clearly shows the purity and the concentration of the DNAs. Plate.1 also presents the base bands of the agarose gel electrophoresis of the genomic DNA of the pure bacterial isolates in their various band lane, it also shows the absorption spectrum of the genomic DNA of the various isolates from AM1, PIV1, PIV3 and NA4. Which depicts the absorbance record according to their wavelengths after carrying out nanodrop spectrophotometric analysis. Then figure 1 to figure 4 shows the evolutionary relationship of the four isolates AM1, PIV1, PIV3 and NA4 with gene bank closest matches.

Table 4: The physicochemical parameters of mixed substrate before fermentation (BF)

Sample	pH	Temperature ^{o}C	Phosphorous Mg/kg	Magnesium %	Potassium Mg/kg	Iron Mg/L	Nitrogen Mg/kg
Watermelon peel and spent mushroom substrate	5.88	29.9	1.265	48	154.70	819	0.854

Table 5: The physicochemical parameters of mixed substrate after fermentation (AF)

Sample	pH	Temperature ^{o}C	Phosphorous Mg/kg	Magnesium %	Potassium Mg/kg	Iron Mg/L	Nitrogen Mg/kg
Biofertilizer	7.39	29.9	0.790	27.6	93.21	929	0.317

Table 6: The physicochemical parameters of planting soil sample.

Sample	P^H	Temperature ^oC	Phosphorous ug/kg	Magnesium %	Potassium g/100g	Iron Mg/L	Nitrogen %
Soil	2.99	28.6	0.1498	21.06	375.17	0.036	8.34

Physicochemical Properties of AF and BF Samples and Planting Soil

Table 4 to table 5 shows the physicochemical parameters of the substrate before fermentation (BF), the liquid biofertilizer after fermentation (AF) and the planting soil sample. It clearly shows that there is an increase in the P^H of the substrate after fermentation which is as a result of the microbial activities going on in the finished product and of course the fermentation process impacted on the decrease and increase of other parameters such as nitrogen, phosphorous, potassium and iron content. But for the soil analysis, it show very low P^H value and reduced amount of available nutrients which indicates that truly there is a great need for fertilization to increase plant growth and high crop yield in the agricultural sector.

Figure 5 shows the growth level of the various experimental set ups after fertilizer application, the results depicts that the set up with liquid biofertilizer application competed favourably with the set up with chemical fertilizer application. This was visible in the shoot length, leave length, leave number, stem girth, leave width and number of branches measurement. This result indicates that the LBF which is eco-friendly, cost effective and biodegradable can be used in place of the CF.

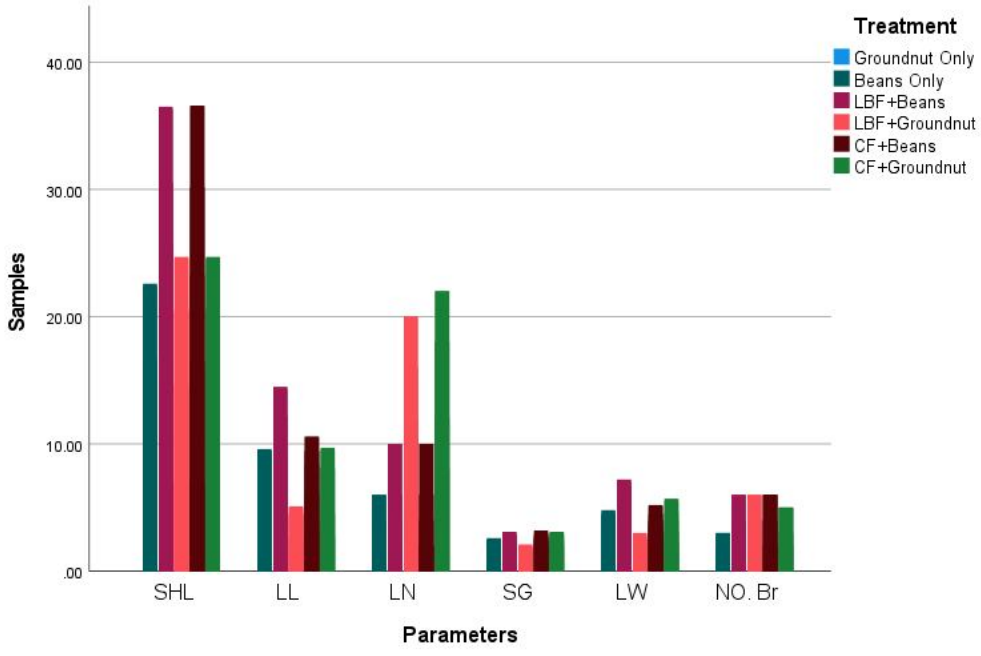

Figure 5 growth level of the various experimental set ups after fertilizer application

LEGENDS:
SHL= Shoot length
LL= Leave length
LN= Leave number
SG= Stem girth
LW= Leave width
No.Br= Number of branches

Figure 6. shows the growth index of various parameters in days after fertilizer application. The result showed that there was a progressive report from the first day of measurement, although there was no growth recorded on day zero.

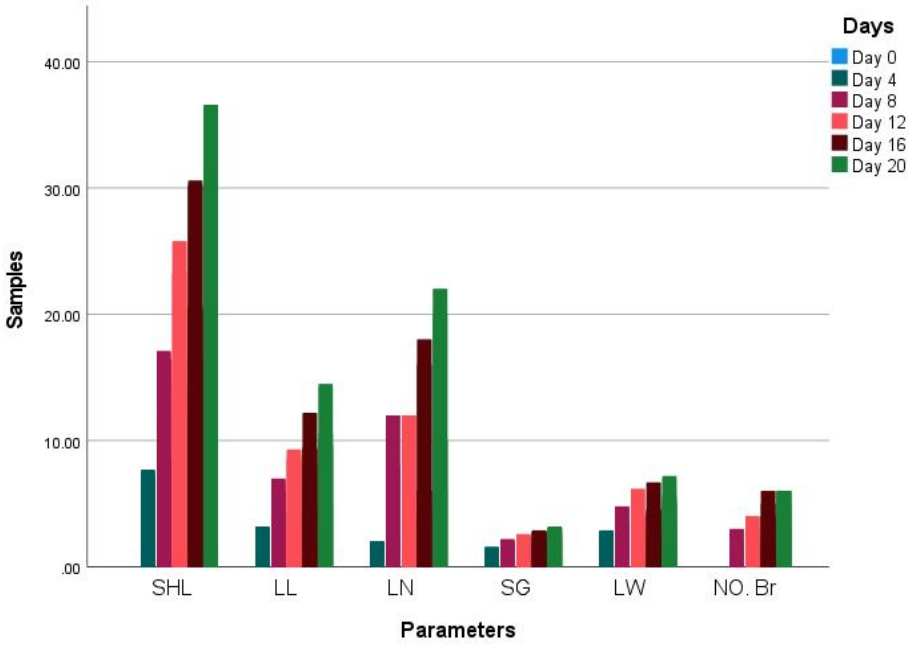

Figure 6: Growth index of various parameters in days after fertilizer application.

LEGENDS:
SHL= Shoot length
LL= Leave length
LN= Leave number

SG= Stem girth
LW= Leave width
No.Br= Number of branches

Figure 4to 6f shows the growth rate of various set ups as measured in separate days, the result showed in figure 4 that there was no measurable growth for LBF + Groundnut, and groundnut only and CF + Groundnut. Then a progressive report was taken from day 4 to day 20. It was on 8th day that the shoot length of LBF+ beans was slightly higher than that of the CF+ beans which is indicative that the liquid biofertilizer promotes plant growth. And on day 20 the growth (shoot length) of chemical fertilizer set up was equal to that of liquid biofertilizer same as their leave length, leave number, stem girth, leave width and no. of branches for the both groundnut set up.

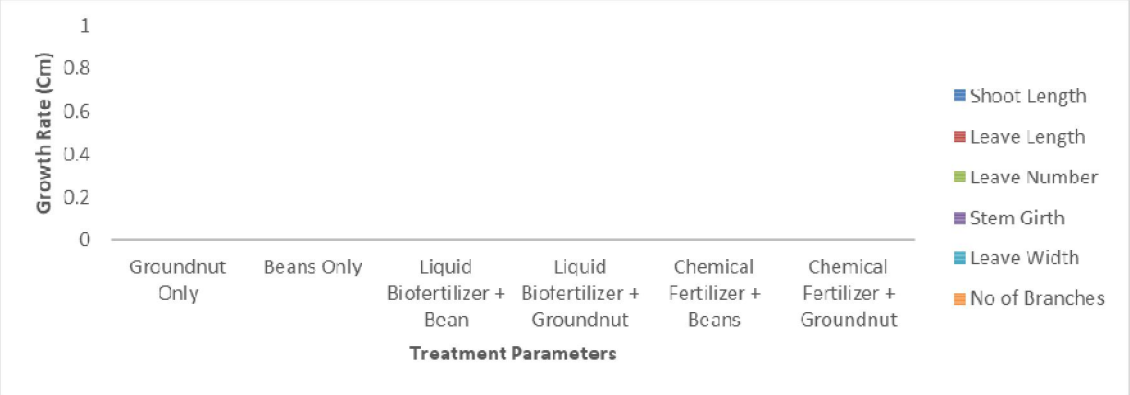

Figure 7: Growth rate of various set ups in day Zero after fertilizer application.

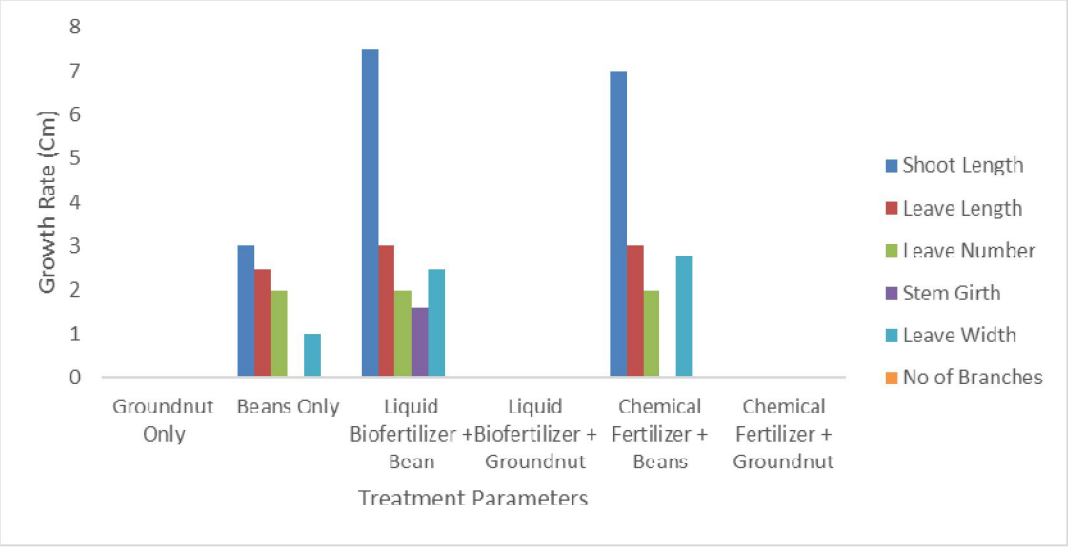

Figure 8: Growth rate of various setups in the day four after fertilizer application

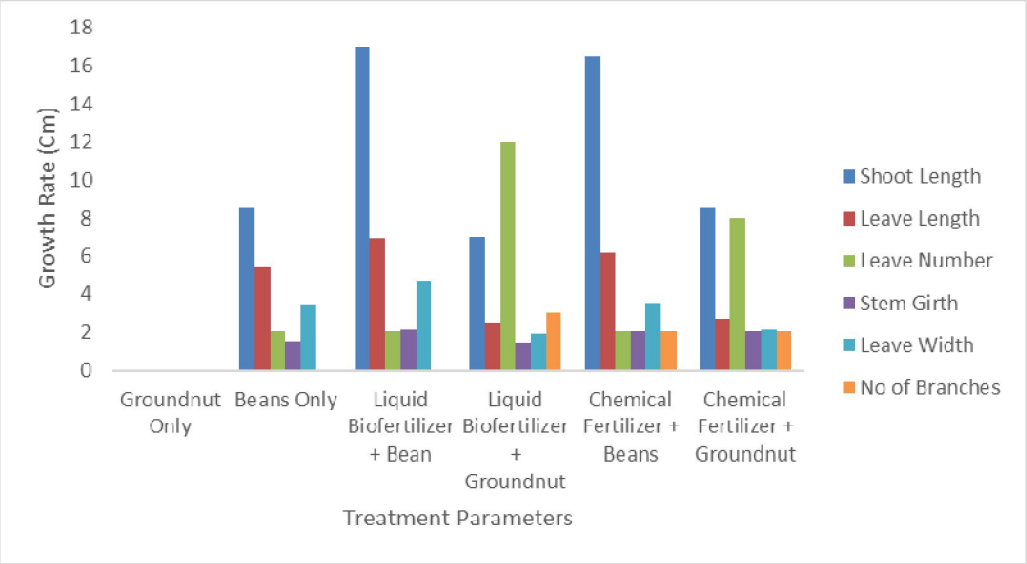

Figure 9: Growth rate of various set ups in day8 after fertilizer application

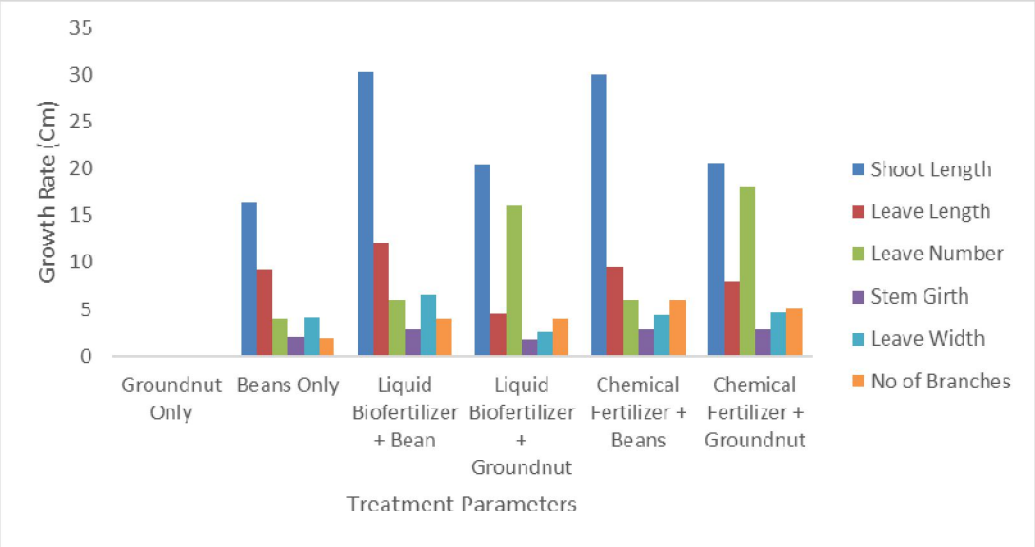

Figure 10: Growth rate of various set ups in day12 after fertilizer application

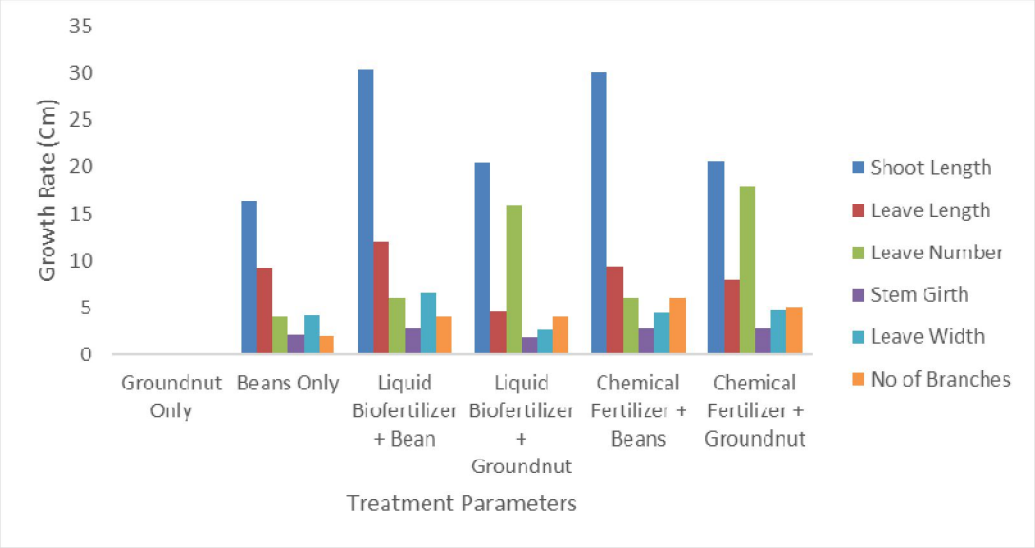

Figure 11: Growth rate of various set ups in day16 after fertilizer application.

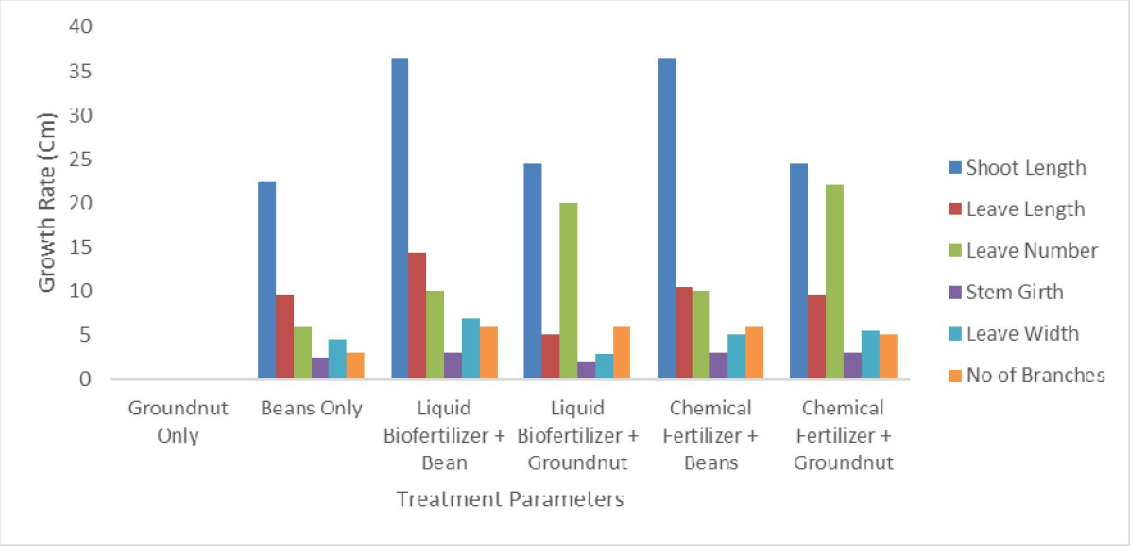

Figure 12: Growth rate of various set ups in day20 after fertilizer application.

Discussion

Soil fertility may be improved with the help of liquid biofertilizer, a natural solution that includes living microbes which increases plant growth through their usual routine activities in the carrier material. The carrier material for this liquid biofertilizer is distilled water.

Table 1. showed the tabulation of some parameters that was used to characterize the tentative organisms, of which most of the isolates were gram negative except NA3 and MSA samples which appeared to be gram positive rod and gram positive cocci. This result is based on the

fact that the substrates for biofertilizer production was decomposed under fermentation process which is an anaerobic Condition. It also showed that *Pseudomonas aeruginosa* is the predominant specie in the liquid biofertilizer which is a gram negative rod, catalase positive, oxidase negative, citrate positive, glucose positive but lactose negative. Wani (2015) states that plant growth-promoting genera *Azotobacter, Bacillus,* and *Pseudomonas* promote plant development and nematode control. These bacteria stimulate plant growth hormones such auxin, cytokinine, gibberellin, and volatile organic compounds. Growth regulators like siderophore fix nitrogen and solubilize organic and inorganic phosphate. The microbiological analysis of the biofertilizer indicated that there is a consortium of microorganisms embedded in this organic product most of which has their origin in the spent mushroom substrate or the watermelon peel and these organisms are environmental organism which has now turned to be beneficial in the environment through the amazing role they play in the biofertilizer.

The molecular analysis revealed the presence of specific microorganisms, as anticipated. These microorganisms including: *Pseudomonas aeruginosa*, *Stenotrophomonas geniculata*, *Sphingobacteriumdaejenense* and *Alcaligenes faecalis* and they are known to play a crucial role in the effectiveness of the liquid biofertilizer. Notably, they exhibit nematicidal activity and have a positive impact on plant growth. Several isolates from these taxa have previously been examined and shown. Nevertheless, the genus Bacillus include several species that are mostly obtained from soil and have characteristics that promote plant growth. Several studies have shown the inhibitory effects of several species, such as *B. cereus, B. subtilis, and B. megaterium*, on plant parasitic nematodes. (Saikia et al., 2013) and Engelbrecht et al. (2018) provided a comprehensive summary of the beneficial role of these organisms as natural predators of root-knot nematodes. They also discussed their ability to kill nematodes and the specific mechanisms by which they operate as biocontrol agents. *Stenotrophomonas* species are a type of bacteria that have a rod shape and are classified as Gram-negative. These bacteria can be found in a wide variety of habitats, including animals and plants. They are very common and can be found in many different types of environments, including extreme ones. However, they are naturally found in the rhizosphere of plants and play a key role in the cycling of sulfur and nitrogen. They are also capable of breaking down complex compounds and pollutants. Additionally, they have a positive effect on plant growth and overall plant health (An and Berg, 2018; Pérez-Martínez et al., 2020). The *Stenotrophomonas* genus thrives in highly specialized environments, including space shuttles, hospitals, and clean rooms (An and Berg, 2018). *Stenotrophomonas* bacteria are gaining attention in study owing to their potential as a very efficient bioinoculant for enhancing plant development and controlling several illnesses in food crops. This feature is gaining increasing biotechnological attention (Ulrich et al., 2021). *Stenotrophomonas* spp., as reported by Kumar et al. (2023), have the capacity to solubilize phosphate and potassium, as well as fix nitrogen. This organism also plays a role in rhizoremediation and phytoremediation processes by producing enzymes that facilitate the degradation of polychlorinated hydrocarbons and metals. Gao et al. (2013) observed that *Stenotrophomonas* spp. played important roles in the degradation of geosmin, hexahydro-1, 3, 5-trinitro-1, 3, 5–triazine (RDX), keratin, macrocyclic hydrocarbon, nitrophenol, and phenanthrene. *Stenotrophomonas* species have shown efficacy in soil bioremediation for agricultural purposes, since they can eliminate diverse chemical pesticides, insecticides, and a broad spectrum of environmental contaminants. While discussing the advantages of *Stenotrophomonas* spp., it is important to note that certain species of *Stenotrophomonas*, such as *Stenotrophomonasmaltophilia*, have been identified as human pathogens. This information has been documented by Lira et al. (2017). However, it should be noted that *Stenotrophomonas maltophilia*can only infect individuals with weakened immune systems and underlying health conditions. In addition, *Stenotrophomonas* spp. have

the ability to produce exopolysaccharides, which provide protection against several environmental challenges, including predation, desiccation, and the effects of antibiotics (Limoli et al., 2015). We also have the record of a novel bacteria *Sphingobacteriumdaejeonense* which is an aerobe, mesophilic, rod-shaped bacterium of the family *Sphingobacteriaceae*. It was recognised to be one of the key players in the inhibition of the hatching of a nematode egg according to Rostami et al., (2021).
Another notable bacteria that was identified is *Alcaligene faecalis*, which is a gram negative, rod shaped aerobe that produces siderophore, that it's an environmental organism that is also a human pathogen, but is an effective plant growth promoting bacteria as it functions In fixing iron (Fe) nutrition in plants (Sayyed et al., 2010) all of which was evident in *A. feacalis* inoculated seeds of groundnut over the control.

Biocontrol potential of the various Microorganisms found in the molecular result:
According to Kumar et al. (2023), *Stenotrophomonas* species has an antagonistic impact on plant infections, and antagonistic microorganisms have been used as an alternative to synthetic fungicides to control plant diseases. In many cropping systems, beneficial bacteria boost growth and disease tolerance. Microbial biocontrol agents may increase food crop productivity by solubilizing phosphate, fixing nitrogen, or preventing illnesses (Parnell et al., 2016). Stenotrophomonas may be a biocontrol agent for several plant diseases that harm commercial crops. *Stenotrophomonas* spp. inhibited pathogenic oomycetes, fungi, bacteria, and insects. *Stenotrophomonas maltophilia* and *rhizophilia* may interact nicely with plants (Ryan et al., 2009). The biocontrol capacity of *Stenotrophomonas* spp. has been shown in several crops by suppressing phytopathogen development and secreting antifungal compounds including maltophilin and xanthobaccin. The results are consistent with what Rostami et al. (2021) found.
Again, the statistical result typically showed that there's a significant difference at 0.05 confidence level between the growth rate of different set ups in the experiment and the days of measurement. For the beans (*Phasolous vulgaris L.*) treatment with liquid biofertilizer and chemical fertilizer, there was significant difference in the shoot length from day four to day twenty as it was observed in figure8 during the measurements on day 4 the shoot length of LBF+ Beans was 7.5cm where as that of CF+ Beans was 7.0cm then on day 8, shoot length was 17.1 for LBF+ Beans whereas that of CF+ beans was 16.5 the slight difference continued till day 20 when equal shoot length was observed this is indicating that there was a positive comparison between the both set up.

Also for the leave length analysis, the LBF+ Beans, had the highest leave length on day 20 but it progressed from day 4 with 3.1 cm to 7.0 cm to 9.3 cm to 12.2 cm and then14.5cm but that of CF+ beans on day 20 (figure 10 had measurement of 10.5 cm which is implying that the growth of the LBF set up was flourishing more better than the CF set up.
For the leave number, the both set ups had equal number of leaves on the various days of measurement. Also there was slight difference in the stem girth of both set ups as there was 3.1 cm measurement for LBF+ beans and 3.0 cm for CF+ beans on day 20, but for the Leave width analysis, there was significant difference in both set ups as the CF+ beans set up had leave width of 5.1 cm on day 20 and LBF+ beans set up had the highest measurement of 7.0 cm.
For the groundnut (*Arachis hypogea*) set up, there was no significant difference in the shoot length of the LBF+ groundnut and that of CF+ groundnut except on day 12 (figure 10) when the shoot length of CF+ groundnut was 16.5 cm whereas that of LBF+ groundnut was 14.5 cm. But for leave length, the chemical fertilizer set up for groundnut has a higher leave length than that of the liquid Biofertilizer as CF+ groundnut had 9.5 cm on day 20, LBF+ groundnut

had 5.1 cm leave length on day 20, furthermore the CF+ groundnut had the highest stem girth of 3.0 cm on day 20, while LBF+ groundnut had 2.0 cm stem girth on day 20. This is still pointing towards the fact that the liquid biofertilizer is competing favourably with the chemical fertilizer but in the growth rate of groundnut set up it was observed that chemical fertilizer is performing more than the liquid biofertilizer. Also for the leave width measurement, CF+ groundnut had a higher measurement as we recorded 5.5 cm leave width for CF+ groundnut on day 20, and 3.0 cm for LBF+ groundnut on day 20. Then for the number of branches, it was recorded that CF+ groundnut had five branches. In contrast, the LBF+ groundnut had six branches, insinuating that the liquid biofertilizer is encouraging higher growth and development of the test crop the same way the chemical fertilizer is. In this growth rate parameters analysis, undermining the impact of environmental factors will be an aberration, as light intensity, humidity and temperature are other environmental factors which negatively impact plant growth. Where any of these arenot in adequate proportion, crops will not grow very well, these factors mentioned above are added to soil and fertilization all of which, in their right proportion,enhance plant growth. This research supports the findings of Okolie et al (2019)

From the physicochemical results, it was observed that most measured parameters were reduced after fermentation except for the iron content. Parameters such as Phosphorus which had 1.265 Mg/kg before fermentation, reduced to 0.790 Mg/kg could be due to the presence of some microorganisms in the finished product that is utilizing the phosphorus as a nutrient source. The examination and detection of the final biofertilizer products showed that the quantity of organic matter, nitrogen, phosphorous, potassium, and other trace elements, as well as the number of living bacteria, met or exceeded the requirements. Phosphorus is a vital plant nutrient that directly or indirectly impacts all biological activities. Phosphorus plays a crucial role in essential plant metabolic activities, including photosynthesis, energy transmission, signal transduction, biosynthesis of molecules, and respiration. Soils contain a significant quantity of Phosphorus, in both inorganic and organic forms. However, its availability is a significant constraint on plant development in several ecosystems globally (Raghothama, 2015). This is because the majority of soil Phosphorus exists in an insoluble state, rendering it unattainable for plants.

Also magnesium content reduced from 48% to 27.6%, Potassium reduced from 154.70 Mg/kg before fermentation to 93.21 Mg/kg after fermentation, then Nitrogen reduced from 0.854 Mg/kg before fermentation to 0.317 Mg/kg after fermentation. This reductions also could be attributed to the effect of the fermentation process and inherent activities of denitrifying bacteria, potassium solubilizing and other microbes that are using the available nutrients as carbon source. The physicochemical findings demonstrate that liquid biofertilizer is a rich source of critical macro and micro nutrients for plant development and soil nutrient replenishment.

It was also deduced by Alexieva et al, (2000) that *B. Subtilis*utilises phosphate in the biofertilizer for its growth and lack of phosphorus results to its starvation in the media and will cause it to secret 10-30 fold extracellular enzymes. So the microbial community available in the biofertilizer is a factor to point at when considering the reduction in the macro and micro nutrients available in liquid the biofertilizer. The physicochemical examination of the soil table in figure 4 also revealed a significant difference at a confidence level of 0.05. This suggests that the soil has a considerable impact on the growth rate of the test crops.

The pot experiment showed that plants started sprouting on the third day, and foliar and soil application of liquid biofertilizer and chemical fertilizer through spraying commenced on the

fourth day. The spraying was done in intervals of days, and the parameters measured, which are the shoot length, leave length, leave number, stem girth, leave width, and number of branches, helped to measure the growth rate of the various set-ups. It was seen that seeds with liquid biofertilizer were growing perfectly well as that of chemical fertilizer also, but it was noted that the seed of groundnut planted in the soil alone did not germinate; this could be due to a lack of essential nutrients in the right proportion in the soil and how available they were to the seeds. However, the beans sprouted in the soil but are not growing well compared to liquid biofertilizer and chemical fertilizer. Furthermore, it was noted that insects ate up the leaves of the beans in chemical fertilizer set-up, whereas that of the liquid biofertilizer set-up was not eaten at all, which indicates that the liquid biofertilizer is also serving not just as a plant growth-promoting agent, but also as a biocontrol agent. This is possible due to the consortium of microorganisms in the liquid biofertilizer. Plants display various relationships with soil-dwelling creatures, including ecological possibilities such as competition, exploitation, neutrality, commensalism, and mutualism. In current plant science, most interaction research has mainly concentrated on mitigating the harmful impacts caused by pathogens, such as herbivory and infection. Identifying mycorrhizal fungi and bacteria in nodulated legumes as root symbionts dates back to the latter half of the 19th century (Morton, 1981). In the 1950s, agricultural seeds were treated with bacterial cultures (namely *Azotobacter chroococcum* or *Bacillus megaterium*) to enhance growth and increase crop production (Brown, 2020).
Recalcitrant soil-borne nutrients are metabolized by soil bacteria for plant nourishment. Most nutrients like N, P, and S are trapped in organic molecules in natural environments, making them less accessible to plants. Plants need soil microorganisms like bacteria and fungi to depolymerize and mineralize organic N, P, and S. These microbial cells discharge their contents by turnover, cell lysis, or protozoic predation.

The result of the physicochemical analysis further exposed that there are still leftover beneficial essential nutrients in the waste substrate (which was used for the liquid biofertilizer production) and enhanced soil fertility. So, our biofertilizer production process supports environmental waste management through recycling and reusing vegetable products and other waste components such as spent mushroom substrate. It also contributes to the circular economy as biofertilizer production from agro waste minimizes environmental pollution and reduces the problem of landfills. Also, considering the harmful effect of chemical fertilizer on the environment and health, liquid biofertilizer production cautions the negative impact of chemical fertilizer on water quality and appreciates the nutrient retention capacity of the soil, bringing about lasting soil fertility. As much as biofertilizer production from agricultural waste involves the decomposition and fermentation of organic materials such as vegetable wastes and other crop residues, it encourages agricultural productivity by harnessing the abilities of important beneficial microorganisms available in the agro-waste. So, according to Kiran et al., (2023) we are now creating a closed system supporting agricultural needs, environmental conservation, and sustainability. This research agrees with the observations of Mintallah et al., (2022).

Conclusion

LBFs are environmentally friendly, inexpensive agricultural products that are preferred for soil amendment and plant growth improvement overchemical fertilizer or inorganic fertilizer asstated in the comparative analysis of how well the liquid biofertilizer competed favourably with the inorganic fertilizer in enhancing the growth of plants and increasing soil fertility. The research supports SDG 2, 12 and 13, then Agenda 2063 no. 4, 5 and 7. Furthermore, by producing biofertilizer from cheap substrates and easily available waste materials, this

research encourages environmental sustainability and conservation by recycling or reusing waste materials to make a beneficial product; in the same vein, this research contributes to circular economy by turning waste into wealth.

References

Anjali K,Deepali K., Pramod K. M., Nagendra K. C., (2021) Current Perspective of Sustainable Utilization of Agro Waste and Biotransformation of Energy in Mushroom DOI:10.1002/9781119741503.ch15.

Bahman N, Sina F. A., Shahaboddin S. & Kwok-wing Chau (2019) Spent mushroom compost (SMC) as a source for biogas production in Iran, Engineering Applications of Computational Fluid Mechanics, 13:1, 967-982, DOI:10.1080/19942060.2019.1658644.

Berg, G., Alavi, M., Schmid, M., and Hartmann, A. (2013). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Mol. Microb. Ecol. Rhizosph. 2, 1209–1216. doi: 10.1002/9781118297674.ch116.

Cezary A. Kwiatkwoski, Elzbieta Harasim. Usher food (2021) The effect of bio-fertilization with spent mushroom substrate and traditional methods of fertilization of common thyme (thymus vulgaris L.) yield quality and antioxidant properties of herbal material.

Cheng, F.; Brewer, C.E. (2021) Conversion of protein-rich lignocellulosic wastes to bio-energy: Review and recommendations for hydrolysis + fermentation and anaerobic digestion. Renew. Sustain. Energy Rev., 146, 111167. (CrossRef)

Cheung,P.C.K. (2010)The nutritional and health benefits of mushrooms.Nutr.Bull,35,292–299.(CrossRef)

Engelbrecht G, Horak I, Jansen van Rensburg PJ, Claassens S (2018) Bacillus-based bionematicides: development, modes of action and commercialization. Biocontrol Sci Techn 28:629–653. https://doi.org/10.1080/09583157.2018.14 69000.

Gao, G., Yin, D., Chen, S., Xia, F., Yang, J., Li, Q., et al. (2012). Effect of biocontrol agent Pseudomonas fluorescens 2P24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. PLoS ONE 7:e31806. doi: 10.1371/journal.pone.0031806.

Glick, B. R. (2020). Beneficial Plant-Bacterial Interactions, 2nd Edn. Cham: Springer International Publishing. doi: 10.1007/978-3-030-44368-9.

Hu Y, Mortimer PE, Hyde KD, Kakumyan P, Thongklang N. (2021) Mushroom cultivation for soil amendment and bioremediation. Circular Agricultural systems 1: 11 https://doi.org/10.48130/CAS-2021-0011.

Jangid MK, Khan IM, Singh S. (2012) Constraints faced by the organic and conventional farmersin adoption of organic farming practices. Indian Res J Ext Educ Spec Issue II:28–32.

Kiran G., Harshal S. K., and Vipul R. S. (2023) Production of biofertilizer from Agro-Waste. Intl. Journal of Resesrch Publications and Reviews. Vol. no. 5, pp5724-5728.

Kumar A, Rithesh L, Kumar V, Raghuvanshi N, Chaudhary K, Abhineet & Pandey A. K. (2023) *Stenotrophomonas* in diversified cropping systems: friend or foe? Front. Microbiol. 14:1214680. doi: 10.3389/fmicb.2023.1214680.

Limoli, D. H., Jones, C. J., and Wozniak, D. J. (2015). Bacterial extracellular polysaccharides in biofilm formation and function. Microbiol. Spectr. 3:10.1128/ microbiolspec.MB-0011- 2014. doi: 10.1128/microbiolspec.MB-0011-2014.

Mintallah M. A. A., Safa M. A. A., Zi Xiang K., Christina V. S., Ajit S., Siewhui Chong. (2022) Liquid biofertilizers as asustainable solution for agriculture. Heliyon 8, 2405-8440. https://Doi.org/10.1016/j.heliyon.2022.e12609.

Mohd Hanafi, F.H.; Rezania, S.; Mat Taib, S.; Md Din, M.F.; Yamauchi, M.; Sakamoto, M.; Hara, H.; Park, J.; Ebrahimi, S.S. (2018)Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): An overview. J.Mater. Cycles Waste Manag. 20, 1383–1396. (CrossRef)

Mustapha Nadhim Owaid, Idham Ali Abed, Sajid Salahuddin Saleem A-lsaeedi. (2017) Applicable Properties of the bio-fertilizer spent mushroom substrate in organic systems as byproduct of cultivation of the *pleurotus*.

N. Z. Othman · M. N. H. Sarjuni · M. A. Rosli · M. H. Nadri · L. H. Yeng · O. P. Ying · M. R. Sarmidi (2020) (eds.), Valorisation of Agro-industrial Residues– Volume I: Biological Approaches, Applied Environmental Science and Engineering for a Sustainable Future, https://doi.org/10.1007/978-3-030-39137-92.

Okolie O.C., H. O. Stanley, Frank-Peterside, N. & C. J. Ugboma (2019) The production of liquid biofertilizer from cassava peels and spent mushroom substrates using microbial inoculants.Asian j. of Biotechnology &Bioresource Technology. https://doi:10.9734/AJB2T/45642.

Parnell, J. J., Berka, R., Young, H. A., Sturino, J. M., Kang, Y., Barnhart, D. M., et al. (2016). From the lab to the farm: An industrial perspective of plant beneficial microorganisms. Front. Plant Sci. 7:1110. doi: 10.3389/fpls.2016.01110.

Pereira G.V.D.M., Neto D.P.D.C., Junqueira A.C.D.O., Karp S.G., Letti L.A.J., Júnior A.I.M., Soccol C.R. (2020) A Review of Selection Criteria for Starter Culture Development in the Food Fermentation Industry. Food Rev. Int.36:135–167. doi:10.1080/87559129.2019.1630636. (CrossRef) (Google Scholar)

Philomina K. Igbokwe, Christian O. Asadu, Emmanuel C. Okpe, Sylvanus E. okoro. (2015) Manufacture of biofertilizer by composting sawdust and other organic waste. International journal of novel research in physics, chemistry & Mathematics.

R Z Sayyed, N. S. Gangurde, P. R. Patel, S. A. Joshi & S. B. Chincholkar (2010) Siderophore production by Alcaligenes faecalis and its application for growth promotion in Arachis hypogaea. Indian Journal of Biotechnology Vol 9, pp 302-307.

Raghothama, K. G. (2015). “Phosphorus and plant nutrition: an overview,” in In Phosphorus: Agriculture and the Environment, eds T. Sims, A. N. Sharpley, and M. L. Cabrera (Madison, WI: American Society of Agronomy; Crop Science Society of America; Soil Science Society of America), 2992–2999. doi: 10.2134/agronmonogr46.c11.

Saikia SK, Tiwari S, Pandey R (2013) Rhizospheric biological weapons for growth enhancement and Meloidogyne incognita management in Withaniasomnifera cv. Poshita. Biol Control 65:225–234. https://doi.org/10.1016/j.biocontrol.2013. 01.014.

Sharma A, Singh AV, Johri BN (2013) Functional and genetic characterization of culturable bacteria associated with late phase of mushroom composting assessed by amplified rDNA restriction analysis. Int J Curr Microbiol App Sci 2(6):162–175.

Ulrich, K., Kube, M., Becker, R., Schneck, V., and Ulrich, A. (2021). Genomic analysis of the endophytic Stenotrophomonas strain 169 reveals features related to plant-growth promotion and stress tolerance. Front. Microbiol. 12:687463. doi: 10.3389/fmicb.2021.687463.

Vasilakis, G.; Rigos, E.-M.; Giannakis, N.; Diamantopoulou, P.; Papanikolaou, S. (2023) Spent Mushroom Substrate Hydrolysis and Utilization as Potential Alternative Feedstock for Anaerobic Co-Digestion. Microorganisms, 11, 532. https://doi.org/10.3390/microorganisms11020532.

Wani SA, Chand S, Ali T (2013) Potential use of Azotobacter chroococcum in crop production: an overview. Curr Agric Res 1:35–38.

Xiaoxuan L. L. Y., Wei L., Dailiang Peng, Liheng Z., Le L., Qinchuan X., Hui L., Chaoqing Y., Peng G. (2018) Comparison of country-level cropland areas between ESA-CCI land cover maps and FAOSTAT data. International Journal of Remote Sensing 39 (20), 6631-6645.

Yuwei Hu, Peter E. Mortemer, Kevin D. Hyde, pattanaKakumayan, naritsadaThongklang (2021) mushroom cultivation for soil amendment and bioremediation. Circular Agricultural systems 1:11 https://doi.org/10.48130/CAS-2021-0011.