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Full Length Research Article

WATER HYACINTH (*EICHHORNIA CRASSIPES*) AS AN INOCULUM CARRIER FOR BIOFERTILIZER

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ABSTRACT
This study evaluates water hyacinth as an inoculum source for biofertilizer. Water hyacinth and harboring river were collected from Alape River in Igbokoda Area, Ondo State. Microbiological analyses of the seaweed were performed using conventional and molecular techniques while physicochemical properties of the water sample were determined using standard techniques. Microbial population of water hyacinth plant parts ranged 1.5×10^7 - 3.4×10^9 (CFU/g, 1. 5 x 10^3- 8.7 x 10^6 (CFU/g, 2.0 x10^3 – 1.02 x 10^8(CFU/g) and 4.3 x 10^5 - 4.1 x 10^6 (SFU/g) for total heterotrophic, phosphate solubilizing, nitrogen –fixing bacteria and heterotrophic fungi respectively. *Klebsiella quasipneumoniae* strain 07A044, *Enterobacter cloacae* strain ATCC 23373, *Pantoea dispersa* (LT 969731.1), *Pantoea dispersa* strain R 56-3, *Pseudomonas aeruginosa, Azotobacter* sp*, Azospirillium* sp. and *Rhizobium* sp. *Fusarium* sp, *Alternaria* sp, *Aspergillus niger, Pythium* sp, *Trichoderma* sp, *Rhizocotonia* sp, and *Pestalotiolopsis* sp. were identified. Result revealed a pH of 7.46 and salinity (0.05%), Turbidity (88. 51 mgL^{-1}), DO (5.22 mgL^{-1}) and BOD (6.13mgL^{-1}) with moderate levels of heavy metals. This study concludes that water hyacinth carries diverse types of microbial biofertilizer which can be harvested and prepared into microbial suspension to promote plant growth and increase crop production without jeopardizing environmental and human health.

Keywords: Biofertilizer, Microbial biofertilizer, Microbial inocula, Mycorrhizal biofertilizers, Water hyacinth.

INTRODUCTION
Agriculture is the second mainstay of the Nigeria economy following petroleum. Profitable agriculture and the desire for food security demand the sustainability of soil quality and integrity. Soil fertility is the ability of a soil to sustain plant growth by providing essential plant nutrients and favorable physicochemical and biological conditions as a medium for plant growth (FAO, 2020). Protracted use of soil for agricultural purposes depletes soil nutrients and consequently affects productive agriculture. The application of fertilizer (natural or synthetic) is a means to improve soil fertility for increased crop production (Edgerton, 2009) and sustainable agriculture to meet food demands. The use of chemical fertilizer has the advantage of providing important macronutrients such as nitrogen, phosphorous, potassium and other micronutrients necessary for optimum plant growth (Thomas and Singh, 2019). However, chemical fertilizers may contain chemical substances with potentials to adversely affect the biological and physical characteristics of soil when they build up with consequence on soil quality and fertility (Farina and Hasanpoor, 2015) and can become toxic to plants, animals and human life (Aggani, 2013). Biofertilizer has been identified as an alternative to chemical fertilizer to improve the physical, chemical and biological properties of soil thereby promoting plant growth and increase crop yield for sustainable farming. Biofertilizers are microbial formulations constituted of beneficial microbial strains immobilized or trapped on inert carrier materials that can be employed to enhance plant growth and increase soil fertility **(**Mendes *et al.,* 2011; Aloo *et al.,* 2019**).** Biofertilizer contains living microorganisms which when applied to plant surfaces, promotes plant growth by increasing the availability, supply, or uptake of primary nutrients to the host through natural processes such as nitrogen fixation, solubilizing phosphorus, and stimulating plant growth along with the synthesis of growth-promoting substances (Sneha *et al.,* 2018). The utilization of microbial products has several advantages over conventional chemicals fertilizers not only for the decrease in quantity of chemical fertilizers, but also for better crop yield in sustainable agriculture. Bio fertilizer production is economical, and does not create pollution problems or jeopardize environmental and human health. Anusha (2012) stated that bioferilizers increase physicochemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter. The self-replication of microbes circumvents the need for repeated application. However, Karen (2021) stated the disadvantages of biofertilizer to include loss of effectiveness if the soil is too hot or dry. Excessively acidic or alkaline soils also hamper successful growth of the beneficial microorganisms. Also, the soil must contain adequate nutrients for biofertilizer organisms to thrive and work.
Thomas and Singh (2019**)** reported that bacteria, fungi, and cyanobacteria which have symbiotic relationship with plants are the most important groups of microbes used in the preparation of microbial biofertilizer. The different groups of biofertilizer based on their nature and function (supply of nitrogen and phosphorous) include microbial nitrogen – fixing bacteria, phosphorous solubilizing microbes, mycorrhizal biofertilizers, other mineral-solubilizing biofertilizers, plant growth-promoting microbes **(**Ahemad and Kibret, 2014; Sneha *et al*., 2018; Thomas and Singh, 2019). These bacteria and certain soil fungi such as *Penicillium* and *Aspergillus* bring about dissolution of bound phosphates in soil by secreting organic acids characterized by lower pH in their vicinity (Thomas and Singh, 2019). Other soil-dwelling microorganisms can further be used as mineral-solubilizing biofertilizers to provide apart from nitrogen and phosphorus various nutrients other than such as Potassium, Zinc, Iron, and Copper (Ansori and Gholami, 2015). Besides nitrogen fixing and phosphorus-solubilizing microbes, there are plant growth-promoting microbes that enhance plant growth by synthesizing growth-promoting chemicals. The rhizobacterial plant growth-promoting mechanisms of antagonism against phytopathogenic microorganisms include production of antimicrobial metabolites like siderophores and antibiotics, gaseous products like ammonia, and fungal cell wall-degrading enzymes which cause cytolysis, leakage of ions, membrane

disruption, and inhibition of mycelial growth and protein biosynthesis (Idris *et al*., 2007; Lugtenberg and Kamilova, 2009). Decomposers such as *Trichoderma viridae, Aspergillus niger, A. terreus, Bacillus* spp. and several Gram-negative bacteria including *Pseudomonas*, *Serratia, Klebsiella*, and *Enterobacter* produce compost biofertilizers by the decomposition of wide variety of materials like straw, leaves, cattle-shed bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, factory waste, etc. (Boulter *et al*., 2002). Soil fertility remediation can be achieved by the use of non-traditional organic materials such as weeds as an inoculum carrier. Water hyacinth (*Eichhornia crassipes*) is a free-floating freshwater weed of the family Pontederiaceae. It is a true water plant and floats by means of spongy petioles. It is the most prolific and spectacular plant of all the aquatic plants that has proven to be a significant economic and ecological burden; clogs up rivers, waterways and entire lakes and obstructs electricity generation, irrigation, navigation, and fishing (Jafari, 2010, Jagadish *et al*., 2012). Water hyacinth is prevalent in tropical and subtropical water bodies where nutrient levels are often high due to agricultural runoff, deforestation and insufficient waste water treatment (Darius *et al.,* 2013). Therefore, this study seeks to evaluate the prolific water hyacinth as an inoculum source for biofertilizer in order to convert its environmental and economic nuisance to agricultural and economic value.

MATERIALS AND METHODS

Collection of samples

Fresh water hyacinth (*Eichhornia crassipes*) leaf, stalk and root and river harboring hyacinth were collected in sterile ziploc bag and bottle respectively from Alape River on latitude 4° 40'5° 00'N and 6° 00' 6° 20'E in Ilaje Local Government Area of Ondo State, Nigeria, labeled and transported immediately in ice chest to the Microbiology Laboratory, for analysis.

Microbiological analysis of Water hyacinth

Enumeration of bacterial and fungal population

Microbial population was determined by the plate count method. Five hundred milligram (500 mg) of each water hyacinth plant parts was rehydrated with 50 ml sterile distilled water in a 100 ml conical flask. Serial dilution of the extract was carried out to 10^8 in a set of test tubes, each containing 9.9 ml sterile distilled water. One (1 ml) of each of the serial dilution was pour plated out in triplicate and overlaid with 20 ml of sterilized nutrient agar (Hi Media), potato dextrose agar (Hi Media), Pikovskaya medium (Gaur, 1981), yeast extract mannitol agar medium (Subba, 1994), Okon medium (Okon *et al*., 1977) and Ashby mannitol agar (Subba, 1994). For the enumeration of total heterotrophic bacteria, total heterotrophic fungi, phosphate solubilizing bacteria, *Rhizobium*, *Azospirillium* and *Azotobacter* respectively. The culture plates were allowed to set and incubated at 35°C for 24 h for nutrient agar and at 28°C for days for the selective media (Suliasih and Widawati, 2005). The fungi plates were incubated at 28°C for 72 h (Firehun *et al.,* 2017). Culture plates in which the number of colonies were 30-300 and its triplicates for each sample was selected and counted. The average count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (CFU/g and spore forming unit (SFU/g) for bacteria and fungi respectively.

Characterization of bacterial isolates

Isolates were purified and identified by colonial and morphological characteristics. Gram staining and biochemical tests including catalase, oxidase, coagulase, spore staining, urease, motility, Voges-Proskauer, nitrate reduction, urease, citrate utilization, H_2S production, sugar fermentation were carried out using the methods of Fawole and Oso (2004) and Holt *et. al.* (1994) was used as reference.

16S rRNA identification of bacterial isolates from water hyacinth

DNA Extraction

Genomic DNA extraction was carried out with column- based JENA bioscience Bacterial DNA Preparation Kit following manufacturer's instructions. Bacteria cells were harvested from 500µl aliquot broth culture using a microcentrifuge at 10,000 g for 1min. The residual pellet was re-suspended in 300µl of Resuspension Buffer and 2µl of Lysozyme Solution. The mixture was homogenized by inverting several times thereafter incubated at 37°C for 1 hour. Resuspended cells were recovered by centrifugation and lysed by adding 300µl of lysis. Buffer after which 2µl RNase A and 8µl proteinase K solution were added; followed by incubation at 60 °C for 10mins. The tube was cooled on ice for 5min. 300µl binding buffer was added to the mixture and vortexed briefly; the mixture was cooled on ice for 5mins and thereafter centrifuged at 10,000g for 5 min. The supernatant was transferred directly into the spin column and centrifuged at 10,000g for 1min to trap the DNA. The trapped DNA was washed twice with washing buffer after which elution buffer into a clean eppendorf tube.

Polymerase Chain Reaction (PCR): 16S rRNA Amplification:
Each PCR reaction mixture consisted of 12.5µl mastermix (2x JENA Ruby hot start mastermix), 1µl (10pmol) each of forward primer 27F 5'AGA GTT TGA TCM TGG CTC AG3' and reverse primer 1492R-5' TAC GGY TAC CTT GTT ACG ACT T 3'1µl DNA template and 9.5µl sterile nuclease free water to make up a total reaction volume of 25 µl. PCR amplification was carried out in an Applied Biosystem 2720 Thermocycler. The mixture was subjected to an initial denaturation at 94°C for 3min; followed by 35 cycles of denaturation at 94°C for 45s, annealing at 55°C for 60s and extension at72°C for 60 seconds; and a final extension at 72°C 10mins.

Gel Electrophoresis:

PCR products were visualized on a 2% agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0) using blue led trans-illuminator.

Sequencing

PCR products were purified and sequenced by Sanger sequencing method using AB1 3730XL Sequencer.

Phylogenetic Analysis

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (- 1283.15) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances

estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (15 categories (+G, parameter = 0.0671)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 25 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 768 positions in the final dataset. Evolutionary analyses were conducted in MEGA X3.4 (Kumar *et al.,* 2018).

Identification of fungi isolates

Fungal isolates were characterized and identified by cultural feature and microscopic observation in lacto-phenol cotton blue and examined under a microscope at 40x objective to detect fungal spores and other fungal structures Leslie and Summerrell (2006)

Analysis of physicochemical properties of water harboring water hyacinth

Physicochemical properties of the water sample from the river were determined using standard physical and chemical analytical techniques. These parameters include temperature, pH, electrical conductivity (EC), turbidity, salinity, total hardness, alkalinity, total dissolved solids (TDS), total solid (TS), total suspended solid (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chloride, sulphate, nitrate and heavy metals which include Cadmium (Cd), Lead (Pb), Lickel (Ni), Copper (Cu), Chromium (Cr), Manganese (Mn), Zinc (Zn), Arsenic (As) and Iron (Fe). Temperature, pH, Conductivity were determined *in-situ* using an ordinary mobile thermometer (Collehhamp England) and Hanna microprocessor pH meter standardized with a buffer solution pH 4 and 9 (Shalom *et al.,* 2011). Conductivity was done using a Jenway conductivity meter (4510 model). Turbidity was determined using Lamotte 2020 portable turbidity meter. TS, TDS and TSS were determined by the gravimetric method (Nsi *et al*., 2020). Other physicochemical parameters were analyzed following standard methods as described by (APHA, 1998; Ademoroti, 1996). The water sample was digested using concentrated nitric acid HNO_3 in order to reduce organic matter interference and convert metal to a form that can be analyzed by AAS. Standard stock solutions were prepared from the digested water sample and then analyzed for the concentration of Iron, Copper, Nickel, Cadmium, Chromium, Lead, Arsenic, Manganese and Zinc using atomic absorption spectrometer (AAS) according to standard analytical procedures by Akoto *et al*. (2018).

Statistical Analysis

Microsoft Excel software was used to compute the mean and standard deviation.

RESULTS

The population of total heterotrophic bacteria and fungi from water hyacinth leaf, stalk and root were in the range of 1.5×10^7 - 3.4×10^9 (CFU/g and 4.3×10^5 - 4.1×10^6 (SFU/g) for bacteria and fungi respectively. The water hyacinth leaf harbors the highest population (3.4×10^9 CFU/g) of THB than the root and stem while the root harbors the highest population (4.1×10^6 SFU/g) of fungi than the leaf and the stem as shown in table 1

The total population of bacterial type of microbial biofertilizer from water hyacinth plant parts on various selective media in table 2 below shows the range of 1.5×10^3- 8.7×10^7 (cfu/gm) and 2.3×10^5- 2.6×10^7 (cfu/g), 3.5×10^6- 1.02×10^8 (cfu/g) and 2.0×10^3- 2.8×10^6 (cfu/g) for PSB on PKV and nitrogen fixing bacteria on Ashby mannitol agar, Okon medium and yeast extract mannitol agar medium respectively.

Table 1: Population of total heterotrophic bacteria and fungi from water hyacinth plant parts (leaf, stalk and root).

Sample	THB (CFU/g)	THF at 28^{O}C (SFU/g)
WHL	$3.4 \times 10^9 \pm 0.2$	$4.0 \times 10^6 \pm 0.4$
WHS	$4.4 \times 10^7 \pm 0.1$	$4.3 \times 10^5 \pm 0.6$
WHR	$1.5 \times 10^7 \pm 0.7$	$4.1 \times 10^6 \pm 0.4$

Data is expressed as mean ± Standard Error

Legend: THB: Total Heterotrophic Bacteria, THF: Total Heterotrophic Fungi, WHL: Water Hyacinth Leaf, WHS: Water Hyacinth Stalk, WHR: Water Hyacinth Root

Table 2: The total population of microbial fertilizer from water hyacinth plant parts

	PSB (CFU/g)	Nitrogen fixing bacteria (CFU/g)		
	PKV	AMA	OM	YEMA
WHL	$8.7 \times 10^7 \pm 1.1$	$2.6 \times 10^7 \pm 0.1$	$1.02 \times 10^8 \pm 0.07$	$2.8 \times 10^6 \pm 1.0$
WHS	$2.4 \times 10^3 \pm 0.06$	$1.7 \times 10^7 \pm 0.6$	$1.87 \times 10^7 \pm 0.1$	$8.3 \times 10^4 \pm 1.5$
WHR	$1.5 \times 10^3 \pm 0.1$	$2.3 \times 10^5 \pm 0.2$	$3.5 \times 10^6 \pm 1.5$	$2.0 \times 10^3 \pm 0.1$

Data is expressed as mean ± Standard Error

Legend: PKV; Pikovskaya, PSB; Phosphate Solubilizing Bacteria, AMA; Ashby mannitol agar, OM; Okon medium, YEMA; Yeast extract mannitol agar, WHL: Water Hyacinth Leaf, WHS: Water Hyacinth Stalk, WHR: Water Hyacinth Root

Microbiological analysis revealed the identity of bacterial isolates from water hyacinth plant parts (table 3) and their distribution among plant parts as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhosa, Klebsiella edwadsii, Azotobacter* sp*, Bacillus* sp*, Pantoea dispersa, Enterobacter cloacae, Azospirillium* sp*,* and *Rhizobium*. Figure 2 reveals the frequency of distribution of eight (8) genera of microbial biofertilizer with *Azospirillium*, *Bacillus*, *Klebsiella* and *Rhizobium* showing 100% abundance.

Figure 1 is the phylogenetic tree showing genetic relationship between 16S ribosomal RNA nucleotide sequences of bacteria isolated from water hyacinth. The phylogenetic tree is based on the alignment of partial 16S rRNA sequences using maximum likelihood method while Table 4 is the complete nucleotide blasts. Results revealed the identities of the bacterial isolates as *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain 07A044, *Enterobacter cloacae* strain ATCC 23373, *Pantoea dispersa* strain R56-3 and *Pantoea dispersa* (LT 969731.1).

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Table 3: Distribution of bacterial isolates from water hyacinth plant parts

Isolate	WHL	WHS	WHR
Klebsiella pneumonia	+	+	+
Staphylococcus aureus	-	+	-
Salmonella typhosa	-	-	+
Klebsiella edwadsii	+	+	+
Azotobacter sp	+	+	-
Pantoea dispersa	strain R56-3	-	+ (LT969731.1)
Enterobacter cloacae	-	-	ATCC 23373
Rhizobium sp	+	+	-
Azospirillium sp	+	+	+
Klebsiella quasipneumoniae strain 07A044	+	-	-
Pantoea dispersa	+		
Ps. aeruginosa	+		
Bacillus sp	+	+	+

Legend: WHL; Water hyacinth leaf, WHS; Water hyacinth stalk, WHR; Water hyacinth root, +; present, -;

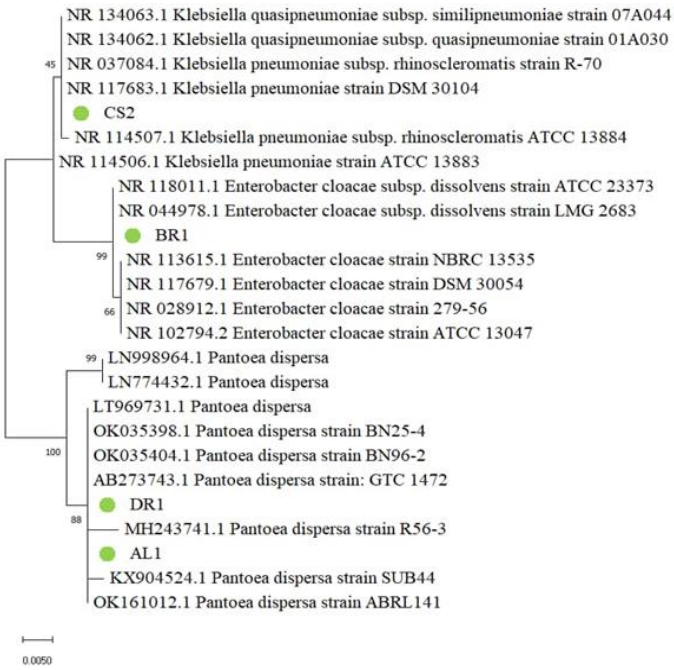

Figure 1: Phylogenetic tree showing genetic relationship between isolates

Legend: AL1: water hyacinth isolate from leaf on Pikovskaya agar, **BR1**: Water hyacinth isolate from root on Ashby mannitol agar, **CS1**: water hyacinth isolate from stem on okon medium, DR1: water hyacinth isolate from root on yeast extract mannitol agar.

Table 4: Complete nucleotide blast of 16S rRNA gene from sample

Isolate code	Source	Organism	Ascension number	% Identity
AL1	Water hyacinth leaf	*Pantoea dispersa* strain R56-3	MH243741.1	88
BR1	Water hyacinth root	*Enterobacter cloacae dissolvens* strain ATCC 23373	NR 118011.1	99
CS2	Water hyacinth stem	*Klebsiella pneumoniae* subsp. *similipneumoniae* strain 07A044	NR 134063.1	45
DR1	Water hyacinth root	*Pantoea dispersa*	(LT 969731.1)	99

Legend: AL1: Water hyacinth isolate from leaf on Pikovskaya agar, **BR1**: Water hyacinth isolate from root on Ashby mannitol agar, **CS1**: water hyacinth isolate from stalk on Okon medium, DR1: water hyacinth isolate from root on yeast extract mannitol agar

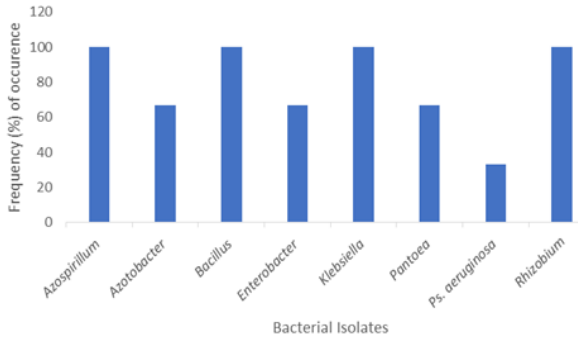

Figure 2: Frequency (%) occurrence of different genera of microbial biofertilizer on water hyacinth plant parts

Identity of fungi isolated from water hyacinth plant parts.
The colonial and morphological characteristics of fungi isolates from water hyacinth plant parts revealed the probable identity of isolates as *Fusarium* sp., *Pythium* sp., *Trichoderma* sp., *Aspergillus niger, Pestalotiolopsis* sp., *Rhizocotinia* sp., and *Alternaria* sp. *Rhizocotinia* sp. and *Alternaria* sp. were found present among all the parts with *Fusarium* only in the stalk.

Table 5: Distribution of fungal isolates from water hyacinth plant parts

Isolate	WHL	WHS	WHR
Fusarium sp.	-	+	-
Pythium sp	-	+	+
Trichoderma sp.	+	-	+
Aspergillus niger	+	+	-
Pestalotiolopsis sp	+	-	+
Rhizoctonia sp	+	+	+
Alternaria sp	+	+	+

Legend: WHL; Water hyacinth leaf, WHS; Water hyacinth stalk, WHR; Water hyacinth root, +; present

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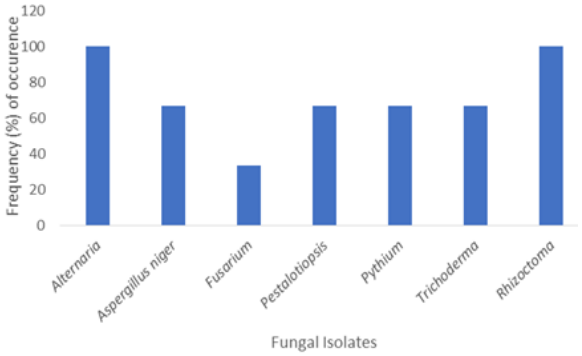

Figure 4: Frequency (%) occurrence of different genera of fungi on water hyacinth plant parts.

The physiochemical parameters of water body harboring water hyacinth.

Table 6a and 6b below show the physicochemical properties of the river sample harboring the seaweed. Result revealed a pH of 7.46 and 0.05% salinity with moderate levels of heavy metals. However, turbidity (88. 51 mgL^{-1}), DO (5.22 mgL^{-1}), BOD (6.13mgL^{-1}) Fe and Mn are in excess of WHO (2008) standard. Among the heavy metals, Fe, Mn and Cu showed highest mean concentrations of 0.472±0.00 ppm, 0.282±0.00 ppm and 0.216±0.00 ppm respectively.

Table 6a: Physiochemical parameters of water harboring water hyacinth

Parameter	Alape Alape
Temp (^{O}C)	26.43333 ± 0.12
pH	7.462333 ± 0.00
Turbidity (NTU)	5.853333± 0.01
Salinity (%)	0.049667±0.00
TH (mgL^{-1})	88.50733±0.01
Alkalinity (mgL^{-1})	50.28367±0.01
Chloride (mg/l)	5.361±0.02
DO (mgL^{-1})	5.223333±0.02
BOD (mgL^{-1})	6.133333±0.15
COD (mgL^{-1})	12.53333±0.15
TDS (mgL^{-1})	90.317±0.01
TSS (mg/l)	2.807333±0.00
TS (mg/l)	93.124±0.01
Electrical Conductivity (μScm^{-1})	178.57
P(ppm)	1.292±0.01
Sulphate(mg/l)	0.592±0.00
Nitrate (mg/l)	0.100±0.00

Legend: BOD = Biological oxygen demand, COD = Concentration of oxygen demand, DO = Dissolved oxygen, TDS = Total Dissolved Solid, TH = Total Hardness, TSS = Total Suspended Solid, P = Phosphorus

Table 6b: Heavy metals of water harboring water hyacinth

Parameter	Alape River
Fe (ppm)	0.472±0.00
Cu (ppm)	0.216±0.00
Ni (ppm)	0.009±0.00
Cd (ppm)	0.002±0.00
Cr (ppm)	0.041±0.00
Pb (ppm)	0.018±0.00
As (ppm)	0.001±0.00
Mn (ppm)	0.282±0.00
Zn (ppm)	0.157±0.01

Fe: Iron, Cu: Copper, Ni: Nickel, Cd: Cadmium, Cr: Chromium, Pb: Lead, As: Arsenic, Mn: Manganese, Zn: Zinc

DISCUSSION

Biofertilizers are microbial formulations constituted of beneficial microbial strains immobilized or trapped on inert carrier materials that can be employed to enhance plant growth and increase soil fertility **(**Aloo *et al.,* 2019; Mendes *et al.,* 2011**).** One of the biofertilizer quality determinants is the type and abundance of microbial inocula. A carrier material is any material that contains microbial inoculum**.** The number of total bacteria, fungi, nitrogen fixing and phosphate solubilizing bacteria suggests abundance with the highest population of PSB and nitrogen fixing bacteria in leaf and the lowest in root. The counts in this study is in the range of values obtained by Widawati and Suliasih (2001) in their study of population of nitrogen fixing bacteria and phosphate solubilizing bacteria in the rhizosphere. There is however, no significant difference in fungal population in the leaf and root parts. Result of this study revealed the abundance of diverse genera of bacteria and fungi including those that have been classified as microbial biofertilizers. This finding agrees with Thomas and Sing (2019) who reported that the most important groups of microbes used in the preparation of biofertilizer are bacteria, fungi and cyanobacteria which have symbiotic relationship with plants. The isolates *Azotobacter*, *Rhizobium, Bacillus Enterobacter, Klebsiella and Pseudomonas*, *Trichoderma*, *Asperigillus niger*, *Rhizoctonia* were grouped as nitrogen fixing, phosphate solubilizing, phosphate mobilizing and plant growth promoting rhizobacteria This result agrees with Adesemoye (2008) who also reported similar bacteria isolates classified as PGPR in his study on soil. In his study, he reported the following genera; *Pseudomonas, Pantoea dispersa, Enterobacter cloacea, Azospirillum, Azotobacter* and *Klebsiella* sp. The growth of these organisms on nitrogen-free media suggests their potentials in fixing atmospheric nitrogen. The bacteria species identified based on 16S RNA gene sequencing in this study belong

to nitrogen-fixing bacteria and phosphate solubilizing bacteria. This result is consistent with the views of (Patriksha *et al*., 2021) who ascribed that the high activity of nitrogenase and ACC deaminase enzyme that reduces ethylene production in plants allows them to be classified as both nitrogen fixing and phosphate solubilizing bacteria. There are no indications that *Staphylococcus aureus* and *Salmonella typhi* have been implicated as microbial fertilizer. Their presence on the plant can only be indicative of contamination of the harboring river through diverse human activities such as swimming, fishing, defecation etc.

The fungi isolate result indicated high pathogenic fungi associated with water hyacinth. This result is in accordance with the results of several studies conducted in different areas in the world. Several authors (Ray and Hill, 2012; Euloge *et al*., 2016 and Firehun *et al*., 2017) revealed similar fungal pathogens associated with water hyacinth. Several of these mycobiota share a common link with those recorded in the center of origin of the weed, the Amazon River basin (Evans and Reeder, 2001). The fungi besides being pathogens are classified as mycorrhizal biofertilizer. This may be because they help in the decay of the weed. Thomas and Singh (2019) classified *Trichoderma* and *Rhizoctonia* among phosphate solubilizing microbes. Isnawati (2018) asserted that in water hyacinth, *Aspergillus niger* and *A. flavus* found as indigenous microorganism because of its cellulolytic activity.
The abundance of microbial biofertilizer population on the plant implies that the physicochemical properties especially pH (7.45) and salinity (0.05) of the water body harboring the seaweed were conducive for the growth and proliferation of the microorganisms. This result is consistent with the views of Zhu *et al.* (2011) who reported that the salt tolerance of PSB isolates is relatively low. This suggests that these microbial biofertilizer may not thrive well river with high salinity.

The values of the physicochemical parameters of the harboring river are within the range reported by Agboola *et al*. (2008) on tropical water bodies and Ndimele *et al*. (2011) on Ologe Lagoon. The temperature (26.4^{O}C), pH (7.45), EC (178.57 µScm^{-1}), TDS (90.32 mgL^{-1}) among other parameters of water harboring water hyacinth is within the permissible limit for drinking water and survival of aquatic lives (FEPA, 2003; WHO, 2008). The pH (6.5 - 8.5), salinity (< 20mg/L) favor the growth requirements of *E. crassipes* and other aquatic organisms. Low concentration of nitrate and phosphate and sulphate is indicative of low nutrient in the river which may have been absorbed by aquatic plants alongside heavy metals. Ndimele and Jimoh (2011) asserted that water hyacinth has ability to absorb and concentrate metals in water. Furthermore, it can be said that the high densities of *E. crassipes* in this river may be responsible for the depletion of these plant nutrients (phosphates, nitrate and sulphates) from the water.

Conclusions and Recommendation

The use of microbial biofertilizers is key to modern agriculture providing alternative to chemical fertilizers to promote plant growth and increase crop production without jeopardizing environmental and human health. The seaweed *Eichhornia crassipes* (water hyacinth) besides being a nuisance in nutrient enriched water bodies, is a low-cost alternative source of organic fertilizer in plentiful supply. Many species of bacteria and fungi with characteristics of microbial biofertilizer were isolated from *Eichhornia crassipes* (water hyacinth). This study concludes that water hyacinth is a good source or an inoculum carrier of diverse groups of nitrogen-fixing bacteria, phosphate solubilizing bacteria and mycorrhizal biofertilizer which can be harvested as biomass or prepared into microbial suspension as alternative to chemical fertilizers to promote plant growth and increase crop production for sustainable agriculture. This study recommends that the isolates from water hyacinth (*Eichhornia crassipes*) be used singly or in consortium as biofertilizer to evaluate their efficiency in improving soil quality and integrity for productive agriculture

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