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Full Length Research Article

PHYSICOCHEMICAL PROPERTIES OF SOIL AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) FROM LEGUME FIELDS IN KADUNA METROPOLIS, NIGERIA

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ABSTRACT
This study explored the physicochemical properties of soils and characterized the plant growth-promoting rhizobacteria (PGPR) associated with the rhizosphere of leguminous crops cowpea and groundnut in Kaduna Metropolis, Nigeria. The soils examined were largely sandy loam and silt loam, with near-neutral pH and moderate to high nutrient contents, particularly phosphorus and nitrogen, which are critical to plant growth. A total of 32 distinct bacterial isolates were recovered, nine of which demonstrated the ability to grow on nitrogen-free medium, indicating potential nitrogen fixation. These isolates also exhibited other growth-promoting traits such as phosphate solubilization, indole acetic acid (IAA) production, siderophore release, and in some cases, hydrogen cyanide synthesis. The predominant genera identified included *Bacillus*, *Pseudomonas*, *Aeromonas*, and *Micrococcus*, all of which have well-documented roles in enhancing soil fertility and suppressing pathogens. The findings confirm that legume rhizospheres in the study area are reservoirs of functionally diverse and agriculturally beneficial PGPR. This opens up opportunities for developing indigenous, eco-friendly bio-inoculants tailored to local soil and crop needs—an important step toward sustainable agriculture in the region.

Keywords: PGPR, indole acetic acid (IAA), and siderophore production.

INTRODUCTION

Soil health is fundamental to agricultural productivity, especially in Sub-Saharan Africa, where soil fertility decline and nutrient depletion severely limit crop yields (Foth, 1978; Atiyong &Michael, 2022). Beneficial soil microorganisms particularly plant growth-promoting rhizobacteria (PGPR) are gaining attention as sustainable tools to improve soil quality and crop performance (Bhardwaj *et al.*, 2014; Khodadadi &Ghorbani Nasrabadi, 2020).
PGPR are diverse, free-living bacteria that colonize the rhizosphere and enhance plant growth through multiple mechanisms, including atmospheric nitrogen fixation, phosphate solubilization, indole acetic acid (IAA) production, siderophore synthesis, and suppression of soil-borne pathogens (Mhatre *et al.*, 2019; Sayyed *et al.*, 2019). Common PGPR genera include *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Rhizobium*, all of which have been documented to increase yields in crops such as wheat, maize, and soybean (Satapute *et al.*, 2012; Gupta &Pandey, 2019; Aasfar *et al.*, 2021).
Legume rhizospheres such as those of cowpea, groundnut, and soybean harbor symbiotic and associative beneficial bacteria that improve soil fertility and facilitate the establishment of other beneficial microbes (Graham &Parker, 1964; Rabiu, 2017a; Aasfar *et al.*, 2021). This characteristic makes legume soils a rich reservoir for isolating multifunctional PGPR strains.
In Nigeria's Northern Guinea Savannah, particularly in Kaduna Metropolis, legume cultivation is widespread. However, there is limited information on the identity, diversity, and functional potential of PGPR communities associated with these crops (Rabiu, 2017a). Identifying native rhizobacterial strains with nutrient-enhancing and growth-promoting traits is critical for developing location-specific bio-inoculants that can improve crop performance and reduce reliance on synthetic fertilizers (Rabiu, 2017a; Ferreira *et al.*, 2019; Kalayu, 2019).
Therefore, this study aimed to characterize plant growth-promoting rhizobacteria (PGPR) and assess the physicochemical properties of soils from legume fields in Kaduna Metropolis, Nigeria, with the goal of identifying native bacterial strains capable of enhancing soil fertility and promoting crop growth.

MATERIALS AND METHODS

Study area

Kaduna metropolis lies in the central part of 46,053 square kilometres of Kaduna state, north western geopolitical zone with the vegetation with in the northern guinea savannah agro-ecological zone of Nigeria. The city comprises of four Local Government council areas namely Chikun, Igabi, Kaduna north and Kaduna south. It lies on latitude 10.609319, 10º 36'33.5484"N and longitude 7.429504, 7º25' 46.2144"E. The rainy season in the state is characterised as warm, oppressive and overcast that usually last for about seven months with the average peak period around July and August. The mean annual rainfall in the state is between 880mm to 1380mm (Omonijo, 2014).

Sample Collection and Preparation

Bulk soil samples were collected from the top layer around the root following the procedure described by Rabiu (2017b), with slight modifications. A total of eight (8) samples were collected legumes rhizosphere soils for cowpeas and groundnut plants. Using a sturdy shovel, 250 g soils were collected at a slant depth of up to 30 cm. The soils collected together with their associated root systems, was placed into clean polyethylene bags, and transported to the Microbiology Laboratory at Kaduna State University for analysis.

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Soil Preparation
A 25 g portion of the sample that is closely associated with the root system is taken for rhizobacteria isolation. For physical and chemical analyses, a portion of each soil sample was spread evenly on a clean, disinfected laboratory bench and allowed to air-dry under ambient conditions for 72 hours. Afterward, visible root fragments were carefully removed, and the soil was gently ground and passed through a sterile 2 mm mesh sieve to obtain a uniform particle size, in accordance with standard soil microbiology protocols.

Soil Particle Size Distribution

Particle Size Distribution
Soil particle size distribution was determined following the method adopted by Bouyoucos (1927). The technique used Calgon (sodium hexametaphosphate) as a dispersing agent to separate the soil particles, and the suspension variation was measured over a one-hour period to assess the relative proportions of sand, silt, and clay in the samples.

Soil Moisture Content Determination
The moisture content of the soil was determined as a percentage of the total soil weight, following the procedure described by the International Institute of Tropical Agriculture (IITA, 1982). Five grams of air-dried soil were weighed and placed in an oven set at 105°C, where they were dried overnight. The loss in weight after drying was used to calculate the moisture content.

Determination of pH and Electrical Conductivity
The pH and electrical conductivity of the soil were determined in a 50ml glass beaker using a pH meter and conductivity meter calibrated with 4, 7, and 9 buffer solutions.

Determination of Soil Total Nitrogen
Determination of Soil Total Nitrogen
The total nitrogen content of the soil was determined using the Kjeldahl digestion method, as described by the International Institute of Tropical Agriculture (IITA, 1982). This method involved digesting the soil sample to convert organic nitrogen into ammonium, which was then distilled and titrated to quantify the total nitrogen content.

Determination of Available Organic Phosphorous
The amount of soil extractable phosphorous was determined using a UV spectrophotometer and employing the sodium bicarbonate extraction technique (Maclean, 1965). Absorbance was taken at 880 nm and compared with the standard curve which have been prepared using 10, 20, 30, 40, and 50 ppm concentration to determine the concentration of the organic phosphorous.

Determination of Soil Organic Carbon
The organic carbon composition of the soil was determined using chromic acid oxidation method developed by Walkley &Black (1934) and Ramamoorthi &Meena (2018).

Enumeration of Plant Growth-Promoting Rhizobacteria (PGPR)
Twenty-five grams (25 g) of the rhizosphere soil prepared by removing the roots of the associated plant was suspended in 500 ml beaker containing 225 ml sterile distilled water to obtain a soil suspension of concentration 1/10 (w/v). This suspensions tenfold serially diluted using sterile distilled water. An aliquot of 0.1 mL was spread plated on 20 ml of solidified Yeast Extract Mannitol Agar (YEMA) and then incubated at 37 ^{0}C for 48 has adopted by Sultana *et al.* (2020). Discrete colonies ranging from 30 to 300 were enumerated and expressed as colony forming unit per gram CFU/g.

Isolation of Plant Growth Rhizobacteria Culture
Bacteria colonies with visibly distinct features were selected and purified by sub-culturing on freshly prepared Yeast Extract Mannitol Agar plates. The pure cultures obtained were then stored on nutrient agar slants at 4 ^{0}C until used.

Morphological and biochemical Characterization of Plant Growth-Promoting Rhizobacteria (PGPR)
The colonial characteristics of the bacterial isolates on Yeast Mannitol Agar was carried out by visual observation for colour, size, texture, elevation, margin and opacity according to the standard procedure (Graham and Parker, 1964; Rabiu, 2017b). The morphological features of motility, and endospore formation and Gram stain were then carried out following conventional bacteriological procedure.
Biochemical tests (indole test, urease test, catalase test, Voges–Proskauer (VP) test, methyl red (MR) test, and citrate test) of the isolate were conducted according to methods by Janda &Abbott (2002) and Javoreková *et al.* (2020).

Plant Growth-Promoting Potentials of Bacterial Isolates
Bacterial isolates were screened for plant growth-promoting characteristics, including Nitrogen Fixing Bacteria, solubilization of phosphate, production of siderophores, Production of Phytohormones (IAA), and Production of Cyanide as described below.

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Screening for Nitrogen Fixing Bacteria
Screening of bacterial isolates for Nitrogen Fixing Bacteria was carried on on nitrogen-free medium as adopted by. In this study, pure bacterial cultures were suspended in 1 ml sterile distilled water and its 0.1 ml aliquot was inoculated on SM basal medium (Per liter) Na_2HPO_4 4.5 g, KH_2PO_4 1.5 g, NH_4Cl 0.3 g, $MgSO_4.7H_2O$ 0.1 g, $Na_2S_2O_3$ solution 100 ml [10 g in 100 ml distilled water] and trace metal solution 5.0 ml (per liter composition trace metal solution: EDTA 50 g, $ZnSO_4.7H_2O$ 22 g, $CaCl_2$ 5.54 g, $MnCl_2.4H_2O$ 5.06 g, $FeSO_4.7H_2O$ 4.99 g, $CoCl_2.H_2O$ 1.61 g, $CuSO_4.5H_2O$ 1.57 g and $(NH_4)_6Mo_7O_{24}$. $2H_2O$ 1.1 g) (Loganathan &Nair, 2004) and incubated at 37°C for 48 hrs. The cultures obtained were transferred on nitrogen free medium LGI medium (per liter) $CaCO_3$ 1.0g, K_2HPO_4 1.0 g, $MgSO_4.7H_2O$ 0.2 g, $FeSO_2.7H_2O$ 0.1 g, $Na_2MoO_4.2H_2O$ 5.0 mg, Sucrose 5 g and bromophenol blue solution 5 ml (Add bromophenol blue 0.5 g to 100 ml of 0.2 N KOH) incubated at 37 °C for 24 to 48 hours and maintained on yeast extract mannitol agar (YEMA) medium (Cavalcante &Dobereiner, 1988; Singh *et al.*, 2013; Aasfar *et al.*, 2021).

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Production of Phytohormones (IAA)

Indole acetic acid, production potentials of the bacterial isolates was determined as described by Duca *et al.* (2014) and (Islam *et al.*, 2016b). Pure bacteria cultures were then inoculated in Luria Bertani broth supplemented with 5 µg/ ml tryptophan and incubated at 30 °C for 5 days.

After incubation cultures were separated by centrifugation at 3000 rpm at room temperature (27 ± 2 °C) for 30 minutes. Two milliliters of the culture supernatant was taken and mixed with 2 drops of orthophosphoric acid, then four milliliters of Salkowski's reagent (50 ml of 35% perchloric acid + 1 ml of 0.5 M $FeCl_3$) was added The mixture was then incubated in the dark for 25 minutes and observed for the development of pink to red colouration. Development of pink to reddish colour indicates indole-3-acetic acid (IAA) production and the intensity of the color was reported as either +, ++, or +++ (Leveau &Lindow, 2005; Shahab *et al.*, 2009; Islam *et al.*, 2016b).

Determination of Phosphate Solubilisation

Phosphate solubilization was determined as adopted by Gupta et al. (1994). Pure bacterial cultures were point inoculation on Pikovskaya's agar medium containing (per liter): 0.5 g of yeast extract, 10 g of dextrose, 5 g of Ca3(PO4)2, 0.5 g of (NH4)2SO4, 0.2 g of KCl, 0.1 g of MgSO4 ×7H2O, 0.0001 g of MnSO4•H2O, 0.0001 g of FeSO4×7H2O and 15 g of agar. Afterward, the inoculated plate agar were then incubated at 28°C for 3 days, Appearance of clear zones around the colonies indicates phosphate solubilization (Gupta *et al.*, 1994).

Determination of Cyanide Production

Hydrogen cyanide was determined using the Castric (1975) method with a slight modification. The isolates were inoculated on modified nutrient agar (suplemented by 4.4 g of glycine per liter) on a petri dish. Cyanide detection solution (CDS) (0.5 % picric acid and 2.0 % sodium carbonate) was prepared in a 50 ml volumetric. A piece of filter paper impregnated with the CDS was placed on the lid of the Petri dish. The Petri dish was sealed with candle wax to prevent the escape of the cyanide and then incubated for 4 days to observe for the discolouration of the filter paper from orange to brown or reddish brown (Rimsha *et al.*, 2021). Change in color of CDS impregnated filter paper disc observed from yellowish orange to light brown was recorded as (+), brown was recorded as (+ +) and reddish brown as (+ + +) as indication of low, moderate and high HCN production, respectively (Sandikar, 2018).

Production of Siderophore

Siderophore production was assessed using a modified method by Arora &Verma (2017). Chrome Azurol S (CAS) agar plates were prepared by adding 100 ml of CAS reagent to 900 ml of sterilized LB agar. Two bacterial isolates were spot-inoculated per plate, with an uninoculated plate serving as control. Plates were incubated at 28°C for 24 hours and observed for orange halos around the colonies, indicating siderophore production.

RESULTS

Table 1: Physical and Chemical Properties of Soil from Legume Fields in Kaduna Metropolis

Sample /LGA code	Area (location)	Color	pH (H_2O)	Electrical conductivity (ds/m)	Moisture Content (%)	Organic carbon content (%)	Phosphorous Content (mg/kg)	Nitrogen content (%)	Sand content (%)	Silt content (%)	Clay (%)
CKN 1	Malali	Light brown	7.19	0.04	7.12	0.39	21.60	0.21	71.7	20.0	8.3
CKS 1	Tudun wada	Dark brown	7.36	0.12	8.85	1.18	45.2	0.35	20.0	72.6	7.4
CCH	Dan bushiya	Light brown	7.24	0.09	6.20	0.48	11.6	0.40	71.2	22.3	6.5
CIG	Zangon aya	Dark brown	7.36	0.09	9.19	0.99	7.4	0.08	21.4	70.5	8.1
GKN 1	Malali	Dark brown	7.28	0.10	7.80	1.00	19.3	0.14	34.6	51.5	13.9
GKN 2	Malali	Dark brown	7.33	0.06	8.85	1.22	10.97	0.18	20.0	68.9	11.1
GIG	Zangon aya	Light brown	6.96	0.03	5.87	0.40	9.25	0.4	65.7	23.3	11.0
GIG2	Rigasa	Dark brown	7.16	0.07	7.64	1.22	3.64	0.21	25.5	70.0	4.5

Key: C=cowpea, G= groundnut, KN= Kaduna north, KS= Kaduna south, I= Zangon aya, IG= Igabi CH= Chikun

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Table 2: Total Aerobic Rhizobacteria Count of Soil from Legume Fields

Sample/LGA code	Sample Location	Mean Count (CFU/g)
CKN 1	Malali	3.7×10^4
CKS 1	Tudun wada	3.1×10^5
CCH	Dan bushiya	2.1×10^4
CIG	Zangon aya	4.2×10^4
GKN 1	Malali	9.2×10^4
GKN 2	Malali	1.45×10^5
GIG	Zangon aya	3.8×10^4
GIG2	Rigasa	2.13×10^5

Key: the first letter in the sample code indicate the crop while the two letters that follows indicate the local government

Table 3: Discrete rhizobacterial Colonies Isolated From the rhizosphere Soil Samples

S/N	Sample	Location	NO of isolates	Isolates Name
1	CKN 1	Malali	4	CA, CB, CC, CD
2	CKS 1	Tudun wada	3	CE, CF, CG
3	CCH	Dan bushiya	4	CH, CI, CJ, CH,
4	CIG	Zangon aya	4	CI, CJ,CK,CL
5	GKN 1	Malali	4	GA, GB, GC, GD
6	GKN 2	Malali	4	GE, GF, GG, GH
7	GIG	Zangon aya	4	GI, GJ, GK, GL
8	GIG2	Rigasa	4	GM, GN, GO, GP

Table 4: Growth of the rhizobacteria isolates on N-free Minimal medium

Isolates	Source	Growth on N-free Minimal medium
CA	CKN	+
CB	CKN	+
CC	CKN	-
CD	CKN	+
CE	CKS	-
CG	CKS	+
CH	CCH	-
CJ	CCH	-
GA	GKN1	+
GC	GKN1	-
GD	GKN1	-
GE	GKN2	+
GG	GKN2	-
GI	GIG1	-
GJ	GIG1	+
GL	GIG1	+
GM	GIG2	+
GN	GIG2	-
GO	GIG2	-

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Table 5: Phosphates Solubilization Ability of the potential nitrogen-fixing isolates

Isolates	Source	Solubilization Diameter (mm)	Colony Diameter (mm)	Solubilization Index
CA	CKN	10.3	4.10	2.50
CB	CKN	12	5.00	2.40
CD	CKN	7.80	5.00	1.56
CG	CKS	8.90	5.00	1.78
GA	GKN1	13.10	6.10	2.15
GE	GKN2	11	4.20	2.60
GJ	GIG1	-	-	-
GL	GIG1	13.10	6.10	2.15
GM	GIG2	8.7	4.10	2.10

Table 6: Indole Acetic Acid production Ability of the Rhizobacteria with potential nitrogen-fixing ability

Isolates	Source	Indole Acetic acid Production	Absorbance
CA	CKN	+++	0.838
CB	CKN	+++	0.823
CD	CKN	+++	0.899
CG	CKS	+++	0.898
GA	GKN1	++++	0.773
GE	GKN2	-	
GJ	GIG1	+++	0.826
GL	GIG1	++	0.535
GM	GIG2	++	0.663

Key; +++ = high concencentration, ++ = moderate concentration, + = low conc. = no production

Table 7: Qualitative Siderophore Production Ability of the Rhizobacteria Grown on the N-free Minimal media

Isolates	Source	Siderophore Production
CA	CKN	+
CB	CKN	+
CD	CKN	-
CG	CKS	+
GA	GKN1	-
GE	GKN2	+
GJ	GIG1	+
GL	GIG1	+
GM	GIG2	-

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Table 8: Hydrogen Cyanide Production Ability of the Rhizobacteria Grown on the N-free Minimal media

S/N	Isolates	Source	Cynide Production
1	CA	CKN	+
2	CB	CKN	-
3	CD	CKN	-
4	CG	CKS	+
5	GA	GKN1	-
6	GE	GKN2	-
7	GJ	GIG1	+
8	GL	GIG1	+
9	GM	GIG2	-

DISCUSSION

The result of the physico-chemical composition of the soil samples from which the plant growth promoting rhizobacteria were isolated shows that all the sampled soil were either sandy loam or silt loamy soils. The soil color is light brown for those consistent with the properties of the soil structures for sandy loam and a bit dark for the sandy silt loamy soil believed to have slightly increased amount of humus. These characteristics are similar to soil textural properties of the northern guinea savannah region in the north western Nigeria as reported by Tanko (2018), Shobayo *et al.* (2021), Sadiq *et al.* (2021). This result suggest a likely variation in terms of the types of the bacterial community in the soil sample since gram negative bacteria tends to prefer a silt textured soil while gram positive bacteria grows better in a sandy soil (Valle *et al.*, 2022). Even the crops are said to have preference to particular soil texture, for instance in a study on the growth and nodulation of cowpea in Kano, cowpea was to have grown better with more root noodles on sandy loam soil compared to other soil textural class (Rabiu, 2017a) . The slight silt and sand variation occasionally observed in some areas is a reflection of the varying land scape of the sampled area as observed by Sadiq *et al.* (2021). This is a good soil textural class for agronomical purpose and has the capacity to support many gram negative and gram positive plant growth promoting bacteria (Valle *et al.*, 2022). This result slightly varies with the result of the study carried out by Atiyong &Michael (2022) whom study the nature and variation of soil properties

All the soil samples have a relatively neutral pH and moderately high moisture content. This is most likely because samples were collected in the middle of the cropping season which is always characterised by heavy to moderate rainfall as it is mostly the case in the northern guinea savannah. Again the sandy loamy soil texture of the sample area means there is a good water retention capacity (Foth, 1978). This result is in contrast to what was reported by Rabiu (2017b) when she studied the phenotyphic and temperature variations in cowpea in some northern states in Nigeria with similar textural characteristics of mainly sandy loam soil including Kano and Jigawa.

The result of the major soil chemical compositions obtained shows a higher presence of Phosphorous and Nitrogen averaging 11.6 mg/kg and 0.27% respectively and lower concentration of organic carbon (0.69%) compared to the result reported by Magashii &Joseph (2017) when they analyzed the Carbon, Nitrogen and Phosphorus composition of agricultural land in the Institute for Agricultural Research (IAR), Ahmadu Bello University in Samaru Zaria. They reported the concentration of phosphorous to be 4.03mg/kg and the percentage of the Nitrogen to be 0.007%. Their result shows that organic Carbon concentration was at 1.06% (Magashii &Joseph, 2017). This variation is likely the result of enhanced agronomic practice in the research institute as regular farmers are more likely to apply inorganic artificial fertilizers such as the Nitrogen Phosphorous Potassium (NPK) without considering the nature and availability of such soil chemicals on their land. However, the result showed a relatively higher soil organic matter, soil nitrogen and phosphorous composition when compared to what was obtained by Mubeen *et al.* (2021) when they studied the "Effect of plant growth promoting bacteria and drought on spring maize". The over reliance on inorganic fertilizers is likely the reason why there is relative reduction of organic carbon in the soils that is mostly cultivated by small holder or subsistent farmers in the area, a phenomenon in which the consequence has out weight the benefit in the long run (Bhatt *et al.*, 2019). The result of the soil nitrogen content showed a significantly higher Nitrogen concentration in the rhizosphere of the leguminous crops than that of the non-leguminous crops. This increase in Nitrogen concentration will affect the microbial abundance in the rhizosphere and is likely going to alter the distribution and the structure of the bacterial community even in the same soil type (Li *et al.*, 2016; Zhao *et al.*, 2020). The application of these phosphorous fertilizers will greatly be reduced as the use of Plant growth promoting microorganisms to enhanced inorganic phosphorous solubilization on phosphourous rich soil is continuously being exploited (Sindhu *et al.*, 2014).

As expected the mesophilic bacterial load of the soil is high ranging between 3.1×10^5 cfu/g to 3.7×10^4cfu/g The correlation between the number or population of soil rhizosphere bacteria and the total soil organic matter was reported by Theophilus *et al.* (2020) when they studied the microbial population of soils in different land use systems namely : forestry, horticultural land agricultural land and postural lands and found that the microbial load in the soil is reduced with the reduction of the soil organic matter from forest soil to agricultural lands.

The result of recovery and isolation of colonies consistent with that

of bacteria that were discrete and unique based on the cultural appearance shows that many discrete colonies were recovered from the rhizosphere of the leguminous crops. The diversity of microorganisms especially of plant growth promoting nature in leguminous crops has been reported by several studies (Shahab *et al*., 2009; Sayyed *et al*., 2019; Pantigoso *et al*., 2020).

Nine (9) of the nineteen (19) isolates tested were able to grow on nitrogen free minimal media and are therefore potential nitrogen fixing bacteria. This is because nitrogen is essential for cellular growth and only cells capable of synthesizing their own usable Nitrogen using the nitrogenase enzyme can grow in a media devoid of the available Nitrogen. However it is important to stress that the bacteria isolated are free living rhizosphere bacteria not symbiotic endophytes associated with the root nodules of the crops. Occurrence of free living bacteria in the rhizosphere of leguminous crops is also a well-documented phenomena just like the bacterial endophytes in the root nodules (Satapute *et al*., 2012; Sultana *et al*., 2020). The reduction in the application of inorganic fertilizer in these farms could also be an important factor in the determination of the abundance of rhizobacteria in the rhizosphere of the leguminous crops (Kimiti &Odee, 2010).

All the nine potentially Nitrogen fixing bacteria, were able to solubilize the inorganic tri-calcium phosphate in Pikovskaya's media indicating that they can be relied upon to solubilize the abundant inorganic phosphates in the rock soil. The isolates were able to solubilize the inorganic tri-calcium phosphates to a varying degree as indicated by their solubulization index. This is in agreement with the study of Reyes *et al.* (1999). These organisms were isolated across all the crops in this study, suggesting that phosphate solubilization is a common trait among the nitrogen-fixing bacteria.
All the nine isolates obtained from the leguminous plants were able to produce the plant hormone indole acetic acid except one. This finding is in agreement with that of (Kanaan &Al-Barhawee, 2021) when they tested isolates from *Medicago sarativa* and Lwin *et al.* (2012) when they analyses the indole acetic acid producing ability of isolates from various rhizospheric soils in Mandalay region, Myanmar and found all the eighteen isolates to produce indole acetic acid. Apart from direct impact on plant growth and development indole acetic acid has been shown to greatly influence the surrounding of the plant and has been used to alleviate biotic stress such as weed and herbs control as well as other plant pathogenic organisms (Khan *et al*., 2016; Bunsangiam *et al*., 2021). It also helps in alleviating plant stress associated with heavy metals contamination by increasing the total chlorophyll and carotenoid contents of plant growing under stress by certain heavy metals (Zhou *et al*., 2020).

Most (78%) of the nitrogen fixing bacteria isolated were able to produce siderophore *in vitro*. This is similar to the result obtained by Javoreková *et al.* (2020) when they evaluated the siderophore producing ability of eleven plant growth promoting rhizobacteria isolated from maize rhizosphere and found seven isolates to be positive for siderophore production.

Siderophore producing plant growth promoting rhizobacteria were also isolated from leguminous plants by Sarwar *et al.* (2020) when they screen siderophore-producing PGPRS isolated from groundnut (*Arachis hypogaea* L.) rhizosphere and their influence on iron release in soil. The bacterial siderophore plays an important role in the growth and development of plant by improving the general wellbeing of the plant (Ghazy &El-Nahrawy, 2021), which can be achieved by sequestering the limited available iron in the soil to a form that can be available to the crop and preventing it access by the plant pathogens subsequently preventing there growth (Jošić *et al*., 2015). The preliminary identification of the siderophore producing isolates in this study showed that three are *Bacillus s*pecies two of each *Aeromonas* and *Pseudomonas* species and a single *Micrococcus* specie. Members of these bacterial genera were also reported to produce bacterial siderophore in a study conducted by Kumar *et al.* (2018) and Ghazy &El-Nahrawy (2021). Most of these organisms have been reported to be good plant growth promoting rhizobacteria with siderophore producing ability (Patel *et al*., 2018; Khan *et al*., 2020; Khodadadi &Ghorbani Nasrabadi, 2020). Siderophore producing plant growth promoting rhizobacteria are not only the chelators of iron, but they form part of social evolutionary community of inter dependence with their plant host (Kramer *et al*., 2020). This is because iron deficiency is a major yield limiting factor for many crops and the bioavailability of iron is very low in the soil due to its chemical nature, low solubility and dissolution kinetics (Ferreira *et al*., 2019). Only four of the isolates evaluated for the production of hydrogen cyanide were positive. Two isolates from each of cowpea and groundnut and were able to produce hydrogen cyanide when grown in glycine supplemented nutrient agar. The production of hydrogen cyanide among different bacterial genera of the plant growth promoting rhizobacteria was also shown in an earlier studies on plant growth promoting rhizobacteria conducted in Benin, were all the tested bacterial isolates evaluated were positive for hydrogen cyanide (Agbodjato *et al*., 2015; Pathak *et al*., 2021) including the purple non sulfur bacteria (Gupta &Sinha, 2020). Production of hydrogen cyanide by these bacteria is an indication of their potential biological control activity because several studies have linked the biological control ability of bacteria to the production of hydrogen cyanide. These include Jošić *et al.* (2015) and Sandikar (2018) whom reported that hydrogen cyanide producing *Pseudomonas* sp. has activity against *Fusarium pythium* and other fungal pathogens in plant respectively. However Olanrewaju *et al.* (2017) argued that hydrogen cyanide is not the sole biological control mechanism in most plant growth promoting rhizo-bacteria but only act in synergy to other substances. This does not in any way undermines the importance of hydrogen cyanide producing bacteria in biological control as they were even shown to help in controlling the crown gall and root-knot nematode, *Meloidogyne incognita* (Kang *et al*., 2018; Abd El-Rahman *et al*., 2019), in the control of potential weeds germination.

The cultural, morphological and biochemical identification of the isolates revealed that the isolates were white, medium, and oval colonies that are Gram-positive rods, and able to ferment all the sugar tested with other biochemical characteristics that were consistent with the members of the *Bacillus* genera (Fritze &Claus, 2003; Logan &Vos, 2009). The occurrence and isolation of gram positive plant growth promoting members of the bacilli reported in this study is in agreement with many studies of its kind (Agbodjato *et al*., 2015; Islam *et al*., 2016a; Jimtha *et al*., 2016; Rahmoune *et al*., 2017; Hashem *et al*., 2019). This is because members of these genus can survive in harsh environmental condition for a very long time (Radhakrishnan *et al*., 2017) due to their ability to form a dormant protective resistant spore. Three of the isolates showed

characteristics that were consistent with that of members of the *Aeromonas* genus (Janda &Abbott, 2002). Two isolates were identified as members of each of the genera *Micrococcus*, *Pseudomonas* and *Staphylococcus* according to their cultural biochemical and morphological characteristics. This is an indication of the diversity of microorganisms present in the rhizosphere soil sampled although the diversity is not necessarily indicative of a better plant bacterial interaction in the soil ecosystem as no specific organisms were seen to monopolize any specific function. This observation concur with that of Nannipieri *et al.* (2017) when they study the relationship between the diversity of microorganisms and soil function.

It can be concluded from this study that the rhizosphere of leguminous crops in kaduna metropolis is a potential source of beneficial bacteria capable of imoroving the soil health and plant development. Subsequently, the finding of this study will motivate further research in Kaduna metropolis towards developing and improving environmentally friendly bio-inoculants that can improve the yield and quality of the legumes and many other crops produced through the newly reinvigorated organic farming in the area. This is because free living Nitrogen-Fixing bacteria represent a key source of naturally available Nitrogen in agronomical soils (Aasfar *et al.*, 2021).

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