Isolation and Characterization of Potassium Solubilizing Microorganism (KSM) from the Rhizosphere and Roots of Crops Indigenous to Ihiagwa-Owerri Imo State Nigeria

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Abstract:- The costly environmental degrading chemical fertilizer demands an affordable sustainable eco-friendly alternative: biofertilizer. Isolates from the rhizosphere and root samples of *Abelmoschus esculentus, Manihot esculenta, Musa paradisiaca,* and *Zea mays* were obtained using standard microbiological procedures, screened for potassium solubilization using Aleksandrov medium; and their potassium solubilization index(KSI) determined. Isolates with KSI ≥ 140 were subjected to morphological and biochemical characterization. Discrete colonies obtained were 143. Total bacterial isolates were 111 and 34 fungal. Potassium solubilizing isolates were 20 (13.8%), potassium solubilizing index (KS1) range from 115.4 to 180. Isolates with KSI ≥140 were identified as *Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp., and *Penicillium* sp. The solubilization efficiency order of the isolates was *Bacillus* > *Pseudomonas* > *Aspergillus* > *Pencillium* The application of the isolates as biofertilizer-producing microorganisms will enhance plant access to potassium ion in the soil thereby boosting agro development.

***Keywords*:-** *Biofertilizer, Eco-Friendly, Rhizosphere, Chemical Fertilizer, Solubilization Index.*

I. INTRODUCTION

Potassium stands as the third essential nutrient required by plants. Its involvement in the biochemical and physiological processes in plants includes stomatal regulation, enzyme activation, energy generation, transportation of water and nutrients, among others. The movement of K ions in and out of the guard cells of the epidermal cells is responsible for the opening and closing of stomata. Proper functioning of stomata is essential for photosynthesis since the CO2 needed for the process enters the plant tissues through the stomata. Potassium plays important role in the activation of enzymes, this is pivotal in adenosine triphosphate (ATP) production, a process that is important in regulating the rate of photosynthesis (Marschner, 2012). The transportation of sugars produced in photosynthesis through the phloem to other parts of the plant for utilization and storage demands energy for active transport (Xu et al., 2020). The plant's transport system uses energy in the form of ATP. During K inadequacy, less ATP is available, and the transport system breaks down. Also, the transport of water and nutrients in the plant through the xylem is influenced by the availability of potassium. K is responsible for the activation of the enzyme needed for the synthesis of starch (starch synthetase). Given these roles of K to plants, deficiency of K in plants results in severe loss in yield and quality of crop produced.

Potassium makes up 2.5% of the lithosphere, its soil concentration ranges between 0.04 - 3.0% (Jurgen &Yingwei, 2000). Three forms of it exist in the soil: 90 – 98% soil potassium mineral; unavailable for plant uptake, 1 – 10% non-exchangeable form of soil potassium; predominantly non expanded clay minerals, unavailable to plants, the exchangeable and solution potassium, available to plants (Hu *et al.*, 2006). The non-exchangeable form will release potassium to the exchangeable form when the level of the exchangeable potassium is low.

Some soil microbiota can make available to plants insoluble forms of potassium minerals. These organisms solubilize potassium from insoluble forms like mica, feldspar, and others through the production of organic acids, siderophores, and also capsular polysaccharides. Solubilization of potassium occurs by complex formation between organic acids and metal ions such as Fe2+, Al3+, and Ca2+ (Styriakova et al., 2003). Microbially produced organic ligands, including metabolic by-products, extracellular enzymes, chelates, and both simple and complex organic acids, enhance the dissolution of aluminosilicate minerals or quartz both in field and laboratory experiments (Grandstaff, 1986; Surdam1988). Production of capsular polysaccharides along with organic acid production like tartaric and oxalic acid by the microorganisms leads to solubilization of potassium. There are documentations that organic compounds produced by micro-organisms such as acetate, citrate, and oxalate can increase mineral dissolution in soil (Sheng et al., 2003). These organic matters, at decomposition, produce acids like citric acid, formic acid, malic acid, oxalic acid. These organic acids, in turn, enhance the dissolution of potassium compounds by supplying protons and by complexing Ca2+ ions.

The use of potassium solubilizers in biofertilizers will increase plant uptake of potassium, this can increase crop production. Microorganisms like *Aspergillus niger*, *Bacillus extroquens,* and *Clostridium pasteurianum* were found to grow on muscovite, biotite, orthoclase microclase, and mica *in vitro* (Archana et al., 2013). In a study, it was reported that potassium solubilizing bacteria *B. mucilaginosus* can solubilize rock K mineral powder such as micas, illite, and orthoclases through the production and excretion of organic acids (Ullaman, 1996).

The work aims to isolate and characterize potassium solubilizing microbes from the rhizosphere and roots of indigenous crops to Ihiagwa-Owerri Imo State Nigeria.

II. MATERIALS AND METHODS

2.1 Sampling Collection

Forty rhizospheric soil and 40 roots were sourced from farmlands located at Ihiagwa-Owerri, latitude 5°25'26" N, longitude 7°1'31" E. Ten rhizospheric samples (soil and root) were sourced from an assortment of *Abelmoschus esculentus*(okro), *Manihot esculenta*(cassava), *Musa paradisiaca* (plantain), and *Zea mays*(maize) plants. The samples were aseptically dug out from the depths 10-30 cm into sterile polythene bags after the crests of the soils were cleared of debris with a clean sterile trowel (Philippot et al., 2012). The samples were transported to the laboratory at 4^0C temperature.

2.2 Sample Preparation and Microbial Isolation

Composite sample from ten soil samples of each plant type was subjected to ten-fold serial dilution and an aliquot (0.1ml) of dilution 10-2 was each spread plated on nutrient agar (NA) and sabrourand dextrose agar (SDA) plates and incubated at 30 ± 2°C for 2-7days. Discrete colonies were stored in slants for further studies (Philippot et al., 2012).

Root samples were surface sterilized, macerated, and subjected to ten-fold serial dilution, inoculation, and incubation as the soil samples (Philippot et al., 2012).

2.3 Screening of potassium solubilizing microorganisms (KSM)

Aleksandrov medium {1% glucose, 0.05% MgSO4.7H2O, 0.2% CaPO6, 0.01% CaCO6, 0.0005%FeCl3, 0.5% potassium aluminium silicate (usual mica), and 3 % agar, with pH-6.5} was utilized for the screening KSM. Sterile Aleksandrov plates were each spot inoculated with loopful 48 hours old bacterial isolates and 3 days needle scrap of fungal isolates at the center and incubated at 30±2°C (Prajapati and Modi, 2012). Colonies showing halo zones were taken as evidence of K-solubilization. The isolates with a clear halo zone were purified three times on Aleksandrov medium. Nutrient agar slant and fungal isolates on SDA slant were used to maintain purified bacterial and fungal isolates respectively.

2.4 Determination of Phosphate Solubillization Index (KSI)

Qualitative estimation of K-solubilization was done by measuring the KSI. Loopful of each isolate (48hours bacterial and 3 days fungal) was spotted on the Aleksandrov medium and incubated at 30±2 °C for 5-7 days in three replicates, with the sterile medium serving as a control. KSI formula, KSI = C+H/C, (C = Colony diameter; H =Halo zone diameter) (Pathak *et al*., 2017). Isolates with KSI ≥140 were preserved for preliminary identification and further studies.

2.5 Preliminary Identification of KSM

Preliminary identification was carried using colonial morphology (the colony colour, colony shape, and elevation aided by hand magnifying glass), gram staining, and biochemical test (citrate utilization, catalase, urease, indole, methyl red, vogues Proskauer, H_2S, sugar fermentation, and nitrate reduction test) for bacteria; and cultural and microscopic characteristics for fungi. The outcome was matched against Bergey's Manual, 9th edition, and atlas of fungi (Behzadi and Behzadi, 2012, Cheesbrough, 2000, and Willey *et al*., 2017).

III. RESULTS

3.1 Results

3.1 Isolates zone of potassium solubilization

The result of the potassium solubilization test is presented in Table 1. The diameter of the isolates zone of clearance on Aleksandrov medium was in millimeter. The symbols (+) was used to represent the diameter of clearance ranging from 0-1.5mm, (++) represent the diameter of clearing ranging from 1.5-3.0 mm, and (+++) represent the diameter of the zone of clearing ranging from 3.0-4.5 mm while above 4.5mm diameter is represented by four pluses (++++). The various abilities of these isolates to solubilize mica, the insoluble potassium content of the medium were the reason for the clearance. Isolates that could not solubilize mica, do not produce a zone of clearance. They are represented by a negative (-) symbol. A total of 145 discrete isolates were obtained from the eighty samples (forty soil samples, forty root samples). Twenty (13.8%) were potassium solubilizers. Out of the 20 isolates able to solubilize potassium, 8(40%) solubilization ability was represented by one (+), 6(30%) solubilization ability was represented by two (++), 4(20%) was represented by three (+++) and 2(10%) was represented by four (++++).

TABLE 1: ISOLATES ZONE OF POTASSIUM SOLUBILIZATION

ZONE OF SOLUBILIZATION	ISOLATES
+	PRZS -1, ORZS-11, MRZS-2, CRZS-3, CRZS-12, MRZS-10, ORTS-7, CRTS-12
++	CRZS-5, PRZS-14, PRZ-38, ORZS-19, MRZS-13,MRTS-3
+++	PRTS-5, CRZS-20, ORZS-13, MRZS-18
++++	ORZS-18, CRZS-23
-	CRZS-1,2,4,6-11,13-19,21,22,24,25
	PRZS-2-13,15-31
	ORZS-1-10,12,14-17,20-27
	MRZS-1,3-9,11,12,14-17,19,20
	MRTS-1,2,4-9
	PRTS-1-4,6-9
	CRTS-1-11,13-15
	ORTS-1-6,8-10

KEYS

1. ORTS- OKRO ROOT SAMPLE; CRTS- CASSAVA ROOT SAMPLE; PRTS- PLANTAIN ROOT SAMPE; MRTS- MAZIE ROOT SAMPLE; CRZS- CASSAVA RHIZOSPHERIC SAMPLE; PRZS- PLANTAIN RHIZOSPHERIC SAMPLE; MRZS- MAZIE RHIZOSPHERIC SAMPLE; ORZS- OKRO RHIZOSPHERIC SAMPLE.
2. ZONE OF SOLUBILIZATION RANGING 0.0- 1.5mm =+; 1.5-3.0 =++; 3.0-4.5mm=+++; >4.5mm=++++;

NO ZONE OF SOLUBILIZATION= -

4.2 Potassium Solubilization Index (KSI) of Isolates

The result of the potassium solubilization index of positive isolates is presented in Table 2. The index is the ratio of the diameter of the zone of clearance to the diameter of colonial growth by a hundred. The result values range from 115.4 to 180. The isolates with the lowest index were isolated from the plantain rhizosphere sample (PRZS-1) and okro root sample (ORTS-7). The isolate with the highest index was isolated from the cassava rhizospheric sample (CRZS-23). Forty-five percent (9) of the potassium solubilizers isolated had KSI ≥ 140. These isolates were subjected to further studies.

TABLE 2: Potassium Solubilization Index (KSI) of Isolates

Isolates	Diameter of Zone of Clearance	Diameter of Colonial Growth	KSI ($^{D}/_{d}\ x\ 100$)
PRZS-14	8.0	5.5	145.5
PRZS-1	7.5	6.5	115.4
CRZS-23	9.0	5.0	180.0
ORZS-11	6.0	5.0	120.0
CRZS-3	7.0	6.0	116.7
MRZS-10	8.5	7.0	121.4
CRZS-20	10.0	6.0	166.7
CRZS-5	6.8	5.0	136.0
MRZS-2	6.2	5.0	124.0
PRTS-5	7.3	5.0	146.0
ORZS-18	12.0	7.0	171.4
CRZS-12	6.0	5.0	120.0
MRZS-13	6.8	5.0	136.0
MRTS-3	7.3	5.0	144.0
ORZS-19	8.0	6.0	133.3
ORTS-7	7.5	6.5	115.4
MRZS-18	10.5	7.0	150.0
CRTS-12	6.0	5.0	120.0
ORZS-13	12.5	7.5	166.7
PRZS-38	13.0	9.0	144.4

4.3 Identification of Bacterial Isolates with KSI≥140 using Colonial, Morphological and Biosynthetate Characteristics

The morphological and the biochemical characteristics of the bacterial isolates with KSI≥ 140 were recorded in Table 3. The isolates with KSI≥ 140 were *Pseudomonas* sp and *Bacillus* sp: Their morphological characteristics were recorded based on their size, shape, margin, elevation, and color. Gram stain test was used to confirm the negativity and positivity of the colonies. *Pseudomonas* sp. were found to be gram-negative as they retain the color of the counterstain used while *Bacillus* sp were gram-positive because they retain the color of the primary stain(crystal violet) and were not decolorized by alcohol. Oxidase test was used to differentiate *Pseudomonas* from other gram-negative bacilli while catalase test was used to test the ability of *Pseudomonas* to grow in the presence of oxygen. Other biochemical tests carried out showed that *Pseudomonas* was citrate, gelatin hydrolysis, nitrates, oxidase, and mannitol positive while it was negative for indole, methyl red, urease, Voges Proskauer, maltose, glucose, and sucrose. Also, *Pseudomonas* is a non-spore former, does not produce gas, and does not hydrolyze starch.

On the other hand, *Bacillus* sp was formed to be catalase, citrate, gelatin, nitrate, Voges Proskauer, mannitol, maltose, glucose, and sucrose positive. *Bacillus* showed a negative result for Indole, methyl red, oxidase, and urease. It does not produce gas but it hydrolysis starch and it is a spore former.

TABLE 3: Identification of Bacterial Isolates with KSI ≥140 using Colonial, Morphological and Biochemical Characteristics.

Isolates	**PRZS-14**	**CRZS-23**	**CRZS-20**	**PRTS-5**	**ORZS-18**	**ORZS-13**
Colonial and Morphological Characteristics						
size						
shape						
margin	2mm	3mm	3mm	2mm	3mm	3mm
	Rods	Rods	Rods	Rods	Rods	Rods
Elevation	Smooth edge	Irregular Edge	Irregular Edge	Smooth edge	Irregular Edge	Irregular Edge
Colour	Convex	Flat	Flat	Convex	Slightly	Flat
	Bluish green	Glassy	Glassy	Bluish green	convex	
		Appearance	Appearance		Glassy	Glassy
Pigmentation					Appearance	Appearance
Gram reaction	+					
Biochemical	-	-	-	+	-	-
Test		+	+	-	+	+
Catalase						
Citrate	+					
Gas	+	+	+	+	+	+
Gelatin	-	+	+	+	+	+
hydrolysis	+	-	-	-	-	-
Indole		+	+	+	+	+
Methyl red	-					
Nitrate	-	-	-	-	-	-
reduction	+	-	-	-	-	-
Oxidase		+	+	+	+	+
Spore	+					
Urease	-	-	+	+	+	-
Voges	-	+	+	-	+	+
Proskauer	-	-	-	-	-	-
Mannitol		+	+	-	+	+
Maltose	+					
Glucose	-	+	+	+	+	+
Surcrose	+	+	+	-	+	+
Starch	-	+	+	+	+	+
Motility	-	+	+	-	+	+
Preliminary	+	+	+	-	+	+
Identification	***Pseudomonas***	+	+	+	+	+
	sp.	***Bacillus***	***Bacillus***	***Pseudomonas***	***Bacillus***	***Bacillus***
		sp.	**sp.**	**sp.**	**sp.**	**sp.**

4.4 Identification of Fungal Isolates with KSI ≥ 140 using Cultural and Microscopic Characteristics

The result of the preliminary identification of fungal isolates with KSI≥ 140 is presented in Table 4. The isolates were *Aspergillus* sp and *Penicillium* sp. *Aspergillus* sp. was found to be a powdery colony, with dark brown front colour. The reverse colour was also brown. It has a flatty spread on the surface of the solid medium. Microscopically, *Aspergillus* sp. has septate and branched hyphae with conidia that appeared in chains. *Penicillium* sp. front colour was found to be grey with a large white border and white reverse. It has long branched septate conidiophores consisting of brown-like conidia in chains at the tips of the phialides.

TABLE 4: Identification of Fungal Isolates with KSI ≥ 140 using Cultural and Microscopic characteristics

Isolates	cultural Characteristics	Microscopic characteristics	preliminary identification
MRTS-3	Powdery, dark brown, flatty spread and brown reverse.	Septate and branched hyphae with conidia in chains.	*Aspergillus* sp.
MRZS-18	Grey colony with large white border and white reverse.	long conidiophores consisting of brown like conidia in chains at the tip of the phialides.	*Penicillium* sp.
PRZS-38	Powdery, dark brown, flatty spread on the surface of the solid medium and brown reverse.	Septate and branched hyphae with conidia in chains.	*Aspergillus* sp.

4.5 Potassium Solubilization Efficiency of Isolates on Aleksandrov Agar

The result of the Potassium Solubilization Efficiency of Isolates on Aleksandrov Agar is presented in Fig 1. The bar chart is the mean of triplicate solubilization indexes of the isolates in two days intervals. The bar chart reveals that bacteria isolates were more potassium solubilizing efficiency than fungal isolates. *Bacillus* sp. had more potassium solubilizing efficiency than *Peudomonas* sp. Among fungal isolates, *Aspergillus* sp. were more potassium solubilizing efficiency than *Penicillium* sp.

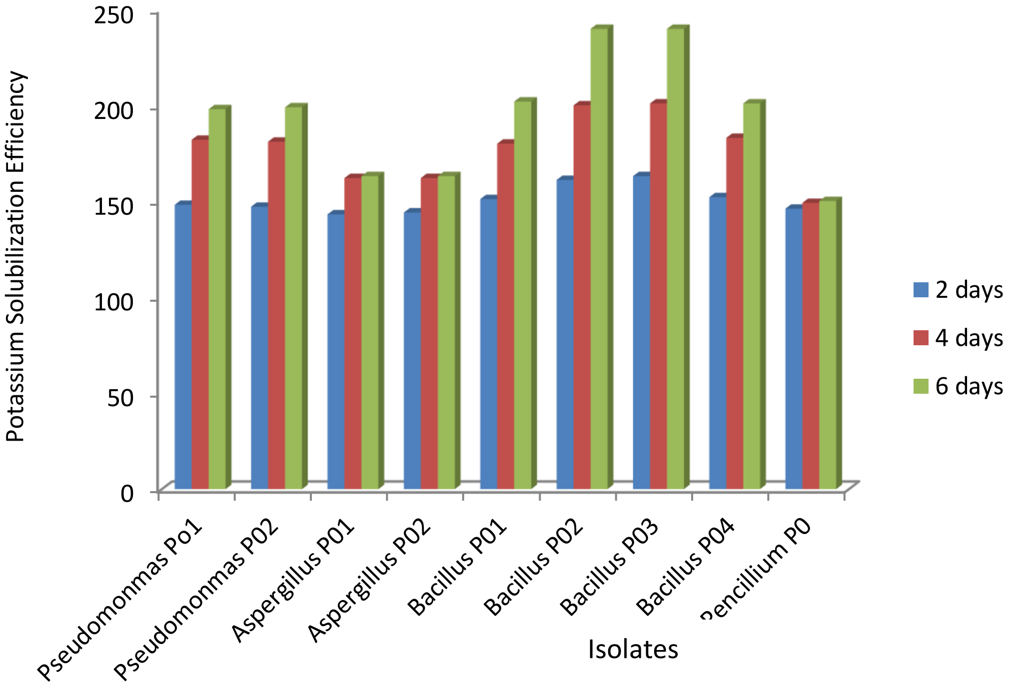

Fig.1: Potassium Solubilization Efficiency of Isolates on Aleksandrov Agar

IV. DISCUSSION

Potassium (K) is the most abundant cation in plant cells. it is the second most abundant nutrient after nitrogen in the leaves (Basak and Biswas, 2010). Most of the potassium in soils exists as insoluble rocks and minerals such as micas, illite, feldspar, and orthoclases. K participates in nutrient transportation and uptake and also confers resistance to both abiotic and biotic stresses. It leads to enhanced production of crops of quality and aids in disease resistance in plants.

Potassium is the third essential nutrient required by plants. It is involved in numerous biochemical and physiological processes in plants like stomatal regulation which is essential for photosynthesis; activation of enzymes involved in adenosine triphosphate (ATP) production; transportation of sugars produced in photosynthesis, through the phloem, to other parts of the plant for utilization and storage. The plant's transport system uses energy in the form of ATP. If K is inadequate, less ATP is available, and the transport system breaks down. Potassium also plays a major role in the transport of water and nutrients in the plant through the xylem. The enzyme responsible for the synthesis of starch (starch synthetase) is activated by K, hence it plays a crucial role in water and nutrient transport.

Crop production is usually severely affected by the deficiency of potassium K. Soil potassium content is huge but largely unavailable to plants. They exist mainly in insoluble forms like mica, feldspar, and others. Potassium solubilizing microorganisms play a vital role in making available insoluble forms of potassium by mineralization. The exploitation of this microbial ability to ensure sustainable agro development void of chemicalization was the reason for the research.

Whitelaw (2000) reported the importance of microbial solubilizers in the maintenance of the global cycle. Alekovsandrov medium was used for the screening of the isolates for potassium solubilizing ability, Aleksandrov medium contains 0.5% potassium aluminum silicate (usual mica), insoluble potassium. The ability of the isolates to solubilize it produces a positive result which manifests as the zone of clearance by 20 (13.8%) of the 145 isolates screened. The production of organic acids by these solubilizers was the reason for the zone of clearance. The carboxylate of the organic acids produced, through chelation and ligand exchange, brought about the solubilization. The diameter of clearance varied between 1.5-4.5mm among the isolates. Out of the 20 isolates able to solubilize potassium, 8(40%) solubilization zone of clearance was ≤ 1.5mm, 6(30%) solubilization was ≤3.0mm, 4(20%) was ≤ 4.5mm and 2(10%) was ≥ 4.5mm. The diameter could be proportional to the concentration of organic acid produced.

The range of potassium solubilization index (KSI) of the isolates was 115.4 to 180. Isolates with KSI ≥ 140 were 45% (9) of the 20 isolates that could solubilize phosphate. Microbial identification protocol carried out on these isolates revealed that 66.7% were bacterial while 33.3% were fungal. The genera of microbes were *Bacillus* sp. were 66.7% of the bacterial isolates while *Pseudomonas* sp. was 33.3%. Fungal isolates were *Aspergillus* sp and *Penicillium* sp, occurring in the frequency of 66.7% and 33.3% respectively.

They solubilize potassium from insoluble by producing organic acids, siderophores, and also capsular polysaccharides. Potassium uptake of plants can be increased by using potassium solubilizers as bio-inoculants further increasing crop production.

These acids enhance the dissolution of potassium compounds by supplying protons and by complexing Ca^{2+} ions. Sheng et al. (2003) reported that organic compounds produced by micro-organisms such as acetate, citrate, and oxalate can increase mineral dissolution in soil. Solubilization of potassium occurs by complex formation between organic acids and metal ions such as Fe2+, Al3+, and Ca2+ (Styriakova*et al.*, 2003). Ahmad *et al.* (2016) stated that potassium solubilizing bacteria *B. mucilaginosus* can solubilize rock K mineral powder such as micas, illite, and orthoclases through the production and excretion of organic acids. It is documented that microbially produced organic ligands include metabolic by-products, extracellular enzymes, chelates, and both simple and complex organic acids enhance the dissolution of aluminosilicate minerals or quartz both in the field and laboratory experiments (Grandstaff, 1986). Sheng and He (2006) reported that the production of capsular polysaccharides and organic acids like tartaric and oxalic acid by the microorganisms leads to solubilization of feldspar and illite to release potassium. Another report showed that potassium was solubilized by the production of inorganic and organic acids and due to the production of mucilaginous capsules containing exopolysaccharides by *Bacillus*, *Clostridium,* and *Thiobacillus (*Groudev, 1987). In another study, the production of organic acids, growth period, and K released in a wild-type strain of *B. edaphicus* and its mutants was assayed. It was found oxalic acid production caused the dissolution of feldspar while oxalic and tartaric acid were involved in mobilizing illite (Hu *et al.*,2006). Sugumaran and Janarthanam (2007) reported the isolation of potassium solubilizing bacteria from Orthoclase, muscovite mica. Among the isolates, *B.mucilaginosus* solubilized more potassium by producing slime muscovite mica. The aforementioned worked identification of *Bacillus* sp. as potassium solubilizer agrees with our result.

The weathering ability of the bacteria involves the production of protons, organic acids, siderophores, and organic ligands. This was seen in *Cladosporoides, Cladosporium,* and *Penicillium* Sp. They have also characterized potassium solubilizing fungi as *Aspergillus terreus* and *Aspergillus niger* based on their colonies and morphology characters. (Prajapati and Modi, 2012). This was in agreement with our result of isolating 66.7% *Aspergillus* sp and 33.3% *Penicillium* sp. Bagyalakshmi *et al.*, (2012) documentation of an *in vitro* study that assessed the potassium solubilization activity by indigenous strains of *Bacillus* sp. *Burkholderia* sp. and *Pseudomonas* sp. at different temperatures, carbon sources revealed that the best carbon source for solubilization of muriate of potash was glucose at 35ºC temperature. In the study of Archana et *al.*, (2013) on rhizosphere soil of different crops from Dharwad and Belgaum districts, a total of 30 bacteria isolates were tested for K solubilization and characterized up to genus level based on morphological and biochemical characters. Out of them, 26 were gram-positive rods belongs to genera *Bacillus* and four were gram-negative rods belongs to genera *Pseudomonas.* This corroborates with our result of having 66.7% frequency of occurrence *Bacillus* sp. to 33.3% of *Pseudomonas* sp. isolates as potassium solubilizers.

V. CONCLUSION

Some microbes, indigenous to Ihiagwa-Owerri exhibit potassium solubilization. The isolates were identified as *Penicillium* sp. *Aspergillus* sp., *Pseudomonas* sp., *Bacillus* sp. The development of biofertilizer products with these organisms will ensure cheap potassium sources, eco-conservation, and sustainable agro development in Nigeria.

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