Abstract: Plant Growth Promoting Rhizobacteria (PGPR) involves the use of large array of soil bacteria to improve yield, plant growth and sustainable food production. As free living and symbiotic rhizobacteria, PGPR exert its role by colonizing extracellular and/or intracellular rhizoenvironment in the quest for carbon source. In the past decades, focus has been on developing a biosafety agro base approach void of continuous burden on soil micro flora as a result of agrochemicals application. However, with clear understanding of PGPR mechanisms of action “biocontrol, biofertilization and biostimulation”, more hope on the possibility of curbing food insecurity amidst rising population has been strengthened. Seeds or soil application of PGPR inoculants enhances phosphates solubilization, biological nitrogen fixation and secretion of plant hormones (indole acetic acid, gibberellins, cytokinins and ethylene) needed for growth and adaptation in stressed environment. As soil pathogen constantly rival the roles of these organisms, PGPR has developed over time wide spectrum of strategies in the form of systemic resistance, iron, space and nutrient competition, antibiotics synthesis, lytic acid production and hydrogen cyanide for efficient food production. In view of this, the review broadens our scope on the use of PGPR as an efficient microbial consortium for enhanced agrobiology and sustenance especially in the tropics where paucity of data on its use, implementation and application of genetically modified organisms has long prevailed.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), Biofertilization, Biostimulation, Biocontrol, Sustainable Food Production

Introduction

The word “sustainable food production” has been an echoing phrase among policy makers, relevant national agencies and international organizations over the past decades. This has become ultimately one of the world most fundamental need to curb food insecurity amidst rising human population (Glick, 2012). Within the last century, man has gradually been faced with the greatest challenge of all time (food insecurity), which has potential of possible looming consequences on the entire human race. Overcoming this confront also will not be an easy task as there has been so much pressure on the natural ecosystem (use of plant for bioethanol) (Chatzipavlidis et al., 2013).

Agriculture has remained the main stream of economic activities within the third world, most importantly in Sub-Sahara Africa. The emergence of mechanized/industrialized agricultural activities has continuously welcomed pollutants in the form of fossil fuel used in powering plants, agro-chemicals, contaminated sewage sludge during irrigation and excessive application of fertilizers. These practices do not only leave an indelible mark on the soil environment but also alters microbial population which aid in plant growth. The use of synthesized agro chemicals/fertilizer has been a point of discuss in public domain in the time past. Though their advantages tend to be immediate, they still present a lasting environmental and public health threat to man through possible entrance of heavy metals via the food chain, death of soil biotic life, environmental deterioration and degradation and alteration or damage of soil structure (Alalaoui, 2007).

Since the inception of microbiological research, only about 1% of the estimated amount of microorganisms has so far been identified. This has left us with large array of microbes that their existence can only be
imagined. These documented organisms live in complex biological environment within which exist interactions arising from other living and non-living influences (Petersen and Klug, 1994). To combat food insecurity through agrobiology, there is need to pay stern attention on the engineering of beneficial microorganisms resident in the soil that has been ascribed with potentials of mitigating associated difficulties in agricultural practices. Thus, suggesting their utilization in environmental cleanup (Van-Veen et al., 1997), renewable energy (Jackson, 1992) and attainment of sustainable agricultural activities (Noumavo et al., 2016).

**Rhizosphere**

The word rhizosphere is referred to the immediate plant root region inhabited by microbial population. This region is host to divers group of microorganisms that are influenced by rich source of nutrients obtain through the root exudates. The subdivision of this rhizosphere into three separate parts ‘exorhizosphere, rhizoplane, endorhizosphere’ was reported by Bowen and Rovira (1999). These regions support healthy competition among organisms for more competency, saprophytic abilities and potential for enhancing plants growth. In addition, its successful organism multiply easily through a broad spectrum of actions as a result of high nutrient and carbon source, favourable competition with other organisms and poses tolerance to stress (Nakkeeran et al., 2005; Ngumbi and Kloepper, 2016). Since rhizosphere is very rich in nutrients, its associate bacteria (rhizobacteria) tend to develop a unique way of communication by enabling of effective selection of its mutual partners, through the creation of host specificity and selective sensitive environment where diversity is less (Sivasakthi et al., 2014).

**Rhizobacteria and Microflora of the Bulk Soil**

Bacteria fund over 95% of the soil microbial activities and are also dominant in abundant. This is as a result of their fast proliferation and ability to utilize a range of nitrogen and carbon source as energy (Glick, 2012). The rhizobacteria concentration in the rhizosphere is estimated to be $10^{12}$ CFU/g (Foster, 1988) while rhizospheric flora which occurs few distance around the root region contains fairly large amount of microbial population $10^6$-$10^7$ CFU/g (Schoenborn et al., 2004; Compton et al., 2010). Under intense environmental stress, rhizobacteria population in the soil ecosystem might be drastically reduced to $10^4$ CFU/g (Timmusk et al., 2011). Microbial structure of the bulk soil flora and rhizobacteria differs with the plant developmental stage, specie type and soil property (Broeckling et al., 2008). Some of the interactions that occur within the rhizosphere and the rhizospheric bulk soil can be said to be neutral, synergistic or antagonistic. The participating genera involved in harmful interaction tend to work against the plant growth, exerting effects in the form of phytopathogen while the beneficial once enhances growth with ability to support nutritional provision (Mahdi et al., 2010; Ahemad and Khan, 2011).

**Plant Growth Promoting Rhizobacteria (PGPR)**

PGPR was first proposed by Kloepper et al. (1980) when he utilized *Fluorescent Pseudomonas* as growth enhancer capable of withstanding plant pathogens. Since then, the term has metamorphosed to include all rhizobacteria capable of directly enhancing plants growth. Recently, it has been used to include wide range of rhizobacteria (*Alcaligenes, Pseudomonas, Azospirillum, Bacillus, Klebsiella, Azotobacter, Enterobacter, Burkholderia, Arthrobacter and Serratia*) that improves plant growth through different mechanisms (Saharan and Nehra, 2011; Haghhi et al., 2011) (Table 1). PGPR exhibit a special role by hindering plant infection with disease, increase nutrient absorption, enhance root and shoot formation, improve seed germination and making the plant more tolerant to most environmental stress (Arora et al., 2008; Lugtenberg and Kamilova, 2009). Interestingly, these organisms have been accrued with fascinating roles ranging from enhanced nitrogen fixation through nodule formation, solubilization of phosphates, production of phytohormones such gibberellins, siderophores, indole acetic acid and serving as low molecular weight agents that modulate plant growth and development (Ma et al., 2009; Odoh, 2017).

**PGPR in Agriculture**

Agriculture is an age long practice. It’s involves the tilling of land and rearing of livestock for food and economic growth. These practices are considered to be the most important human occupation within the tropics (Khan et al., 2014b). Rhizobacteria through the improvement of plant growth, synthesizes some secondary metabolites such as phytohormone, enzymes, siderophores and antibiotics (Noordman et al., 2006; Ahmad et al., 2008), required for plant growth. They help in fixing atmospheric nitrogen, provide nutritional uptake by solubilizing phosphate and producing biologically active molecules (Arshad and Frankenberger, 1992). Studies has shown that for PGPR to be utilized in crop production, it must be able to exert its effects in either one of these three ways; providing the plant with growth-promoting compounds (Glick, 1995), uptake of certain essential nutrients such as phosphorous, nitrogen, sulfur, calcium and magnesium, (Cakmakci et al., 2006; Belimov and Dietz 2000) and averting plants diseases (Khan et al., 2002; Lugtenberg and Kamilova, 2009).
| PGPR                | Mode of action                                      | Plants                  | Outcome                                                                 | References                           |
|---------------------|-----------------------------------------------------|-------------------------|-------------------------------------------------------------------------|---------------------------------------|
| Achromobacter       | Indole acetic acid synthesis and Phosphate solubilization | Improves all round growth performance | Ma et al. (2009)                                                        |
| Azoarcus            | Nitrogen fixation                                  | Rice                    | In situ gradual spread and dominant of inoculants over the plant endophytic life style. | Reinhold-Hurek and Hurek (1998)       |
| Azorhizobium        | Nitrogen fixation                                  | Wheat                   | Increased lateral root formation and development                        | Sabry et al. (1997)                  |
| Azotobacter         | Nitrogen fixation                                  | Wheat, Tobacco, Maize, Coffee | After inoculation on the seedlings                                       | Wani et al. (2013)                   |
| Bacillus            | Antibiotic production                              | Alfalfa                 | Bacillus cultures suppresses alfalfa disease causing agent *P. medicaginis* | Silo-Suh et al. (1994)                |
| Bacillus            | Auxin synthesis                                    | Potato                  | The strain enhances the auxin content of the inoculated plants at more than 400% when compared to the non-inoculated once. |                                       |
| Bacillus            | Cytokinin synthesis                                | Cucumber                | Well-developed lateral roots.                                            | Sokolova et al. (2011)               |
| Bacillus            | Gibberelin synthesis                               | Alnus                   | Peper                                                                    | Joo et al. (2005)                    |
| Bacillus            | Induction of plants stress and resistance          | Peanuts                 | Plant becomes more stress tolerance due to increased soil N, P and K content arising from the inoculants. They also serve as alternative to chemical fertilizer. | El-Akhal et al., 2013                |
| Brevibacillus       | Indole acetic acid synthesis                       | Tomato                  | Efficient in plants and micorrhizal growth even at high metal toxicity   | Vivas et al. (2006)                  |
| Brevic              | Siderophore production                             | Sugar cane              | Increase soil microbial biomass vis-à-vis soil nutrient.                  | Radzki et al. (2013)                 |
| Chryseobacterium    | Siderophore production                             | Tomato                  | Increasing soil microbial biomass                                      | Simonet et al. (1990)                |
| Frankia             | Nitrogen fixation                                  | Maize                   | Egamberdiyeva (2007)                                                    | Muñoz-Rojas and Caballero-Mellado (2003) |
| Gluconacetobacter   | Nitrogen fixation                                  | Sugar cane              | Sheng et al. (2008)                                                     |                                       |
| Kluyvera ascorbata, Microbacterium G16 | Indole acetic acid synthesis and siderophores production | Tomato | Increase soil microbial biomass vis-à-vis soil nutrient.                  |                                       |
| Microoccus luteus, Rhizobium, Bradyrhizobium | Indole acetic acid synthesis and Phosphate solubilization | Non-legumes | Antoun et al. (2004)                                                    |                                       |
| Mycobacterium       | Induction of plants stress resistance              | Maize                   | Egamberdiyeva (2007)                                                    |                                       |
| Peamibacillus       | Indole acetic acid synthesis                       | Lodgepole pine          | Bent et al. (2001)                                                      |                                       |
| Phyllobacterium     | Potassium and phosphate solubilization             | Strawberries            | Aid in phosphate solubilization and plants protection against pathogens  | Flore-Felix et al. (2015)            |
| Pseudomonas         | Antibiotics production                             | Wheat                   | Mazzola et al. (1995)                                                   |                                       |
| Pseudomonas         | ACC deaminase synthesis                            | Mung bean               | Ahmad et al. (2013)                                                     |                                       |
| Rhizobia            | Nitrogen fixation                                  | Legume                  | Young and Haukkka (1996)                                                |                                       |
| Rhizobium           | Hydrogen cyanide                                   | Legume                  | Increase plants biomass due to enhanced nutrient uptake                  | Tham et al. (2011)                   |
| Rhizobium           | Indole acetic acid synthesis                       | Lettuce                 | Flores-Felix et al. (2013)                                              |                                       |
| Rhizobium           | Siderophore production                             | Peper, carot            | Garcia-Fraile et al. (2012)                                            |                                       |
| Streptomyces        | Siderophore production                             | Indian lilac            | Verma et al. (2011)                                                    |                                       |
| Sphingomonas        | Giberellin synthesis                               | Tomato                  | Khan et al. (2011)                                                      |                                       |
| Sinorhizobium       | Chitinase and glucanase production                 | Pigeon pea              | Kumar et al. (2010)                                                    |                                       |

Evident of these are demonstrated in the improved growth and productivity of many commercial crops such as maize (Sandhya *et al.*, 2010), rice (Ashrafuzzaman *et al.*, 2009), black pepper (*Dastager et al.*, 2010), wheat (*Cakmakci et al.*, 2007), sugarcane (*Sundara et al.*, 2002), cotton (*Anjum et al.*, 2007), Banana (*Mia et al.*, 2010) and cucumber (*Maleki et al.*, 2010). There has been public call for possible exploitation of PGPR in biofertilizers production, microbial rhizoremediation (*Odoh et al.*, 2017a) and biopesticides synthesis (*Adesemoye et al.*, 2008) for sustainable environment.
Mechanisms of Action

In recent time, PGPR has been classified based on its direct ability to provide essential compound to plants or indirectly by preventing the deleterious effects of phytopathogenic organisms (Glick, 1995). The direct mechanisms include biofertilization, stimulation of root growth, rhizo-remediation, phytohormones production, plant stress control and efficient uptake of certain nutrients from the environment. Besides reduction of plants disease through antibiotic production, antifungal metabolites, induction of systemic resistance; they also compete favorably with pathogen for nutrients and niches (Pliego et al., 2011; Egamberdieva and Lugtenberg, 2014). In general, PGPR function by preventing plants diseases condition “Biocontrol”, facilitating the uptake of certain nutrients from the environment “Biofertilization” and synthesizing phytohormones “Bistimulants” (Glick et al., 1998). Advances in these field has implicated PGPR in growth promotion of soil stabilizing plants, control flooding, aid plant growth in acidic conditions, and used in phytoremediation technologies (Burd et al., 2000; Zhuang et al., 2007; Odoh et al., 2017b).

Biocontrol

PGPR has been identified as biocontrol agent with the capacity to suppress a wide range of organisms possible of presenting disease condition in plant. For rhizobacterial to be an efficient biocontrol agent against pathogenic bacteria, fungi and viruses, it must utilize one of the following mechanisms; production of antibiotics, competition for nutrients and niche, signal interference, induced systemic resistance, hydrogen cyanide and lytic enzymes production (Podile and Kishore, 2006; Lugtenberg and Kamilova, 2009). Generally, these mode of actions antagonizes fungi, bacteria and nematode as pathogens of interest in their order of severity. Consequently, PGPR control the involvement and application of beneficial rhizobacteria or their metabolites in minimizing the negative impact of pathogens while promoting healthy living in plants (Junaid et al., 2013).

Biocontrol Mode of Actions

The preferential rate of spore-forming Bacillus and other specie by farmers who are recognizing the need for an alternative pest control strategy that is void of environmental damage has been increasing in recent time, primarily due to their long term viability (Borriss, 2015). In characterizing plants associated bacterial; biocontrol bacterial agents usually takes preeminent owing to their ability to suppress phytopathogens for enhanced plant health and reduce harvest loss. Production of antimicrobial secondary metabolites and siderophores and Stimulation of induced systemic resistance (a multifactorial process) is dependent on several compounds produced by the rhizobacteria e.g., c-LP surfactin and volatiles (Raaijmakers et al., 2010). However, a combine effects of these strategies could be necessary for improved crop yield through sound bioformulation of a number of viable microbial living spores (PGPR) and concentrated culture supernatants with antimicrobial metabolites (Borriss, 2015).

Production of Antibiotics

Antibiotics production is one of the most studied biocontrol strategies display by PGPR. Some good examples include amphiisin, 2, 4-diacyltolyploscinol (DAPG), oomycin-A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone and the cyclic lipopeptides synthesis (Loper and Gross, 2007). Basically, these biochemicals are produced by Pseudomonads strains, Bacillus, Streptomyces and Stenotrophomonas sp. As an active chemical agent, they are influenced by biotic and abiotic factors. Antibiotics are low weight molecular compound that suppress the development of plants pathogenic microorganisms. Phloroglucinols (Phl), D-gluconic acid and 2-hydroxymethyl-chrom-4-one have successfully been utilized as biocontrol agent (Kaur et al., 2006; Cazorla et al., 2006; Perneel et al., 2008). Phloroglucinol is a benzenetrol, primarily used in pharmaceutical production of Flopropione (Singh et al., 2010). Phloroglucinols are naturally found in certain plant species and are also produced by soil microorganisms. The 2,4-diacyltolyploscinol (DAPG) is the widely studied phloroglucinol produced by Pseudomonads. Its causes membrane and zoospore damage in Pythium sp. These antibiotics acts as an inhibitor to aldose reductase, an enzyme involved in metabolism of glucose to fructose (Odoh et al., 2017). Phenazine also enhances the survival of bacteria in anaerobic conditions using endogenous phenazines, as withnessed in the survival of P. aeruginosa facilitated by extracellular electron transfer (Wang et al., 2010).

Elsewhere, increase productivity as a result of biocontrol inoculants was reported in S. rochei inhibition of pepper root rot caused by phytopthora (Ezziyyani et al., 2007); S. platensis against R. solani leaf blight/seedling blight of rice (Wan et al., 2008); Tomato wilt and fusarium root rot caused by S. griseoviridis (Minuto et al., 2006); S. hygroscopicus infection caused by Colletotrichum gloeosporioides anthacnose and in wide range of crops (Prapagdee et al., 2008).

Competition for Nutrient and Space

For rhizospheric bacteria to claim dormant over the rest of soil microorganisms, it must be able to compete favourably for the available nutrient and space. This is
required to limit the incidence and severity of plant disease (Kamilova et al., 2005). Consequently, such adaptation makes the root unfit to host pathogens as a result of PGPR fast colonization. As a negative form of association, the most competent group of microorganisms takes charge and controls the whole metabolic activities. Aside the inherent growth which PGPR acquires via competition as a result of sufficient nutrient availability, other properties such as presence of flagellium, lipopolysaccharide, chemotaxis and the usage of secreted root exudates enhances their survival (Lugtenberg and Kamilova, 2009). A good illustration can be seen in unavailability of iron to phytopathogenic fungi when chelated by siderophores synthesized by PGPR. Conversely, iron is one of the essential nutrients required by all microorganisms for synthesis of ATP, formation of heme, reduction of ribotide precursors of DNA and a number of functions (Saraf et al., 2011). In niche competition, a physical occupation of site by PGPR is enhanced through delay tactics by preventing the colonization of pathogens until the available substrate is exhausted (Heydari and Pessarakli, 2010). This feature has been an age long adaptive property that is capable of fighting some pathogenic bacteria, fungi and viruses. This potentially position the plant as strong and highly adapted specie (Van Loon, 2007). The gene and gene product involved in this form of biological control has been poorly documented. Unlike the systemic acquired resistance (SAR) (Handelsman and Stabb 1996), which is a state of defense that is systemic acquired resistance (SAR) (Handelsman and Stabb 1996), which is a state of defense that is

**Induced Systemic Resistance (ISR)**

PGPR triggers inducement of some kind of defense system known as Induced Systemic Resistance (ISR) that is capable of fighting some pathogenic bacteria, fungi and viruses. This potentially position the plant as strong and highly adapted specie (Van Loon, 2007). The gene and gene product involved in this form of biological control has been poorly documented. Unlike the systemic acquired resistance (SAR) (Handelsman and Stabb 1996), which is a state of defense that is activated all through the plant following primary infection by pathogens (Rylas et al., 1996). Induce Systemic Resistance (ISR) utilizes organic acid and plant hormones (salicylic acid, jasmonic acid and ethylene) in signaling and stimulation of the host plant defense response against variety of phytopathogens (Niranjan et al., 2005; Beneduzi et al., 2012; Pieterse et al., 2014). PGPR response to ISR is usually felt by increased physical and mechanical strength of the cell wall as well as adjustment of biochemical and physical reaction to environmental pressure (Labuschagne et al., 2010). ISR in PGPR can be in the form of salicylic acid, siderophores production, lipopolysaccharide, flagella, N-acyl homoserine lactone ( AHL) molecules (Van Loon 2007; Shuhegger et al., 2006) and antibiotics. The participating organisms in this form of biocontrol include B. pumilus, Pseudomonas sp and enterobacteria (Jourdian et al., 2009). In a wider scale, application of PGPR strain as seed coat have improved tremendously the ISR against Colletotrichum lagenarium which causes anthracnose in cucumber, Pseudomonas syringae causing angular leaf spot and bacterial wilt by Erwinia tracheiphila (Zehnder et al., 2001).

**Signal Interference**

For an organism (beneficial or pathogenic) to exert it effects, a particular quorum is required. This requirement especially in gram negative organisms is communicated via a small diffusible signaling molecule called N-Acyl Homoserine Lactone (AHL). This signal interference regulatory agent allows the cells to sense the population of their kind and to express certain character. The development of essential physiological characters such as production of pathogenicity/virulence factors, swarming, swimming, twitching motilities, and rhizosphere colonization can also be credited to cell signaling (Gray and Garey, 2001; Miller and Bassler 2001). The discovery of enzyme capable of degrading AHL is considered to be a fight in the right direction against phytopathogens quorum-sensing system, as B. thuringiensis has shown to efficiently decrease the incidence and development of potato soft rot caused by Erwinia carotovora using signal interference strategies (Dong et al., 2004).

**Production of Lytic Enzymes**

Also, synthesis of extracellular enzymes such as chitinases, β-1-3 glucanases, lipases, cellulases and proteases by rhizobacteria has been suggested to be a vital form of biocontrol (Markovich and Kononova 2003). They are hydrolytic enzymes that degrade wide range of compound usually of plant origin. They are also efficient in the lysing of fungal cell wall (Mabood et al., 2014). Palumbo et al. (2005) has suggested the significant of beta-1, 3-glucanase on the biocontrol activities of Lysobacter enzymogenes strain C3 against Bipolaris leaf spot caused by Phytophthora sp. As multifunctional organic protein, these enzymes form protection from desiccation and against abiotic and climatic factors (Qurashi and Sabri, 2012). Lytic enzyme can be used in the control of blight in pepper by Phytophthora capsici (Jung et al., 2005). Fusarium infection (Hariprasad et al., 2011) and sugar beet by Pythium ultimum (Dunne et al., 1997). Chaiharn et al. (2008) illustrated the antagonistic potential of PGPR through the production of chitinase, β 1, 3 glucanase, proteolytic enzymes and cellulase at low concentration, even as Pseudomonas sp has proven to be a good candidate (Cattelan et al., 1999). Mycorparasitic and Trichoderma species has also been implicated in their antagonistic biocontrol activities against Rosellinia necatrix and other plant pathogens using chitinases (Hoopen and Krauss 2006; Harman et al., 2004).
Production of hydrogen cyanide (cyanogenesis) is predominantly associated to *Pseudomonas* sp. Quantitatively, this can be detected using the techniques described by Lorck (1948). HCN, a volatile biocontrol agent has been well studied. Its cyanide ion inhibits metalloenzymes, principally in copper containing cytochrome c oxidases (Blumer and Haas, 2000). Cyanide produced by *Pseudomonas* strains has successfully been used to curb canker of tomato (Lanteigne et al., 2012). As a secondary metabolite produced by gram negative bacteria, it is formed from glycine and catalyzed by HCN synthase (Castric, 1994). *P. fluorescens* strain CHA0 (Voisard et al., 1989) was used to control tobacco black root rot caused by *Thielaviopsis basicola* (Laville et al., 1998). However, because of the aggressive colonizing strength of *Pseudomonas fluorescens*, it has effectively been used in the control of soil-born plant pathogens (Lugtenberg et al., 2001). There are indications that a good number of rhizobacteria has cyanogetic property when provided with glycine in their growth culture.

**Production of Siderophore**

Iron is a vital element needed by all forms of life. It is one of the most abundant mineral deposits on earth. The unavailability of biological forms of iron for plant utilization creates perplexing circumstances for their growth. Siderophore which means iron carrier or iron chelating is an important strategy developed to increase iron (Fe$^{3+}$) bioavailability as a unique constituent of cytochrome, enzymes co-factor and heme or non-heme proteins. Siderophores are low molecular weight biomolecules produced by microorganisms and has strong affinity with Fe$^{3+}$ ions (Sureshbabu et al., 2016). When Fe is limited, microbial iron chelating agents (siderophores) scavenge and provide plants with Fe from the mineral phase through the formation of soluble Fe$^{3+}$ complexes. Containment of soil borne phytopathogens by siderophore producing *Pseudomonas* has been reported (Buyens et al., 1996). Related studies has shown that siderophore production occurs in both gram positive and gram negative organisms with specific example of *Bacillus, Rhodococcus, Enterobacter* and *Pseudomonas* genera (Tian et al., 2009). Consequently, this property is also exhibited by some plant especially grasses (phyto-siderophores) (Van der Helm and Winkelmann, 1994), as they form constituent in fertilizer formulation, regulate iron intake capacity in plants and facilitate growth (Miller and Malouin, 1994).

One of the major challenges limiting efficient production of siderophore is environmental factors. These include pH, soil iron level, their forms, presence of other trace elements, inadequate supply of carbon, nitrogen and phosphorus (Duffy and Défago, 1999). However, siderophore mediated growth promoting activity of PGPR is associated with the suppression of root pathogens by competitive exclusion. Thus, preventing harmful microorganisms access to environmental iron by extracellular iron complex formation (Podile and Kishore, 2006; Ahemad and Khan, 2011; Saharan and Nehra, 2011). Also, works have shown that PGPR synthesis of siderophore improve not only the growth performance and adaptability in stress condition, but also enhance their ability to absorb both radioactive iron and rhizospheric metals iron even at low concentration (Robin et al., 2008; Dimkpa et al., 2009). Apart from creating favourable competitive room for bacteria against some pathogenic microorganisms by removing iron from the environment (Persello-Cartieaux et al. 2003), chelated iron has also proven to possess one of the weakest bond with fungi (Loper and Henkel, 1999). This condition seems possible considering the fact that many bacterial siderophores differ in their abilities to sequester iron leading to it biological and/or adaptive deprivation of the scarce commodity (iron). Iron chelating has also been linked with potential of promoting bacterial auxin synthesis by reducing the detrimental effects of heavy metals through chelation mechanism (Dimkpa et al., 2008).

**Biofertilization**

This is the application of microbial inoculants on seeds, plant surfaces, or soil to colonize root rhizosphere. This condition enhances growth through the supply and availability of primary nutrients to the plant. Mahdi et al. (2010) defined biofertilization as cultures of bacteria, fungi and algae either alone or in combination, packed in a carrier material. Bhardwaj et al. (2014; Arora et al., 2012) where of the view that biofertilization play a vital role in atmospheric nitrogen fixation, mineralization of organic compounds and phytohormones synthesis. It is an essential components of organic agriculture and vital in maintaining long-term soil fertility and sustainability through the production of safe and healthy food. With current campaign to halt the over dependent on chemically synthesized fertilizers, focus has been on harnessing the potential microorganisms for improved agrobiology (AElzal and Asghhari, 2008; Bhardwaj et al., 2014). Inversely, the use of chemical base fertilizer to enhance soil fertility and crop yield has often negatively impinged on the complexity of both biotic and abiotic matter turnover (Perrott et al., 1992; Steinshamn et al., 2004). This is due to leasing and run-off of nutrients especially Phosphorus (P) and Nitrogen (N) resulting to poor soil quality (Tilman, 1998; Gyaneshwar et al., 2002). Chatzipavlidis et al. (2013) opined that for an efficient formation and utilization of biofertilizer, there must be proper preparation/formulation of the inoculants, selection of adequate carrier and designing
Biofertilization Mode of Actions

Here, a direct mechanism which enhance plants growth through nitrogen fixation and nutrient solubilization has been identified (Sandy and Butler, 2009; Bhattacharyya and Jha, 2012). Biofertilizers are the preparations containing cells of microorganisms which may be N fixers, P solubilizers, S- oxidisers or organic matter decomposers. They are called bioinoculants, which on supply to plants improve their growth and yield. As a bio healthy inoculants containing living cells of different types of microorganisms, they have the ability to mobilize nutritionally important elements from non-usable form through biological stress (Khan and Naeeem, 2011; Mazid et al., 2012). During mycorrhiza colonization, bioactive ligands called Myc factors and Nod factors are secreted by mycorrhiza and rhizobium. The phenomenon is usually facilitated through a communication signal using a transduction pathway (Roberts et al., 2013), thus triggering further transduction pathway signal through some chemical receptors for the release of Ca²⁺ in the cytosol (Sieberer et al., 2009).

Fixation of Nitrogen

Nitrogen (N) is a vital element for all forms of life. It is the most important nutrient for plant growth and also an essential constituent of nucleotides, membrane lipids and amino acids (Marschner, 1995). It constitutes the fourth most important plants dry mass. The biological fixation of atmospheric nitrogen is an important microbial activity for the maintenance of life on earth. This process occur when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase; a highly complex oxygen labile enzyme conserved in free-living symbiotic diazotrophs (Franche et al., 2009). The process coupled with the hydrolysis of 16 equivalents of Adenosine Triphosphate (ATP) is accompanied by the co-formation of one molecule of H₂. Considering the two types of nitrogen fixation (symbiotic and non-symbiotic) base on the plant involved and the associated group of organisms, it is agreed that non-symbiotic bacteria fix lesser amount of nitrogen than the root nodule bacteria (rhizobia) (James et al., 1997). In spite of their low fixing capacity, some PGPR have shown to be very effective in augmenting this process by making the scarce nutrient (nitrogen) available to plants. In the event of non-symbiotic nitrogen fixing activities, free living diazotrophs stimulate the growth of non-leguminous plants. The genera identified in this group include Azorarcus, Azotobacter, Acetobacter, Azospirillum, Burkholderia, Diazotrophicus, Enterobacter, cyanobacteria, Pseudomonas and Gluconacetobacter (Anabaena, Nostoc) (Vessey, 2003; Bhattacharyya and Jha, 2012). While in symbiotic form, bacteria such as Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium and Frankia (a nitrogen fixing Actinomycete), trees and shrubs (Zahran, 2001) exerts their functions. Application of cultures with diazotroph PGPR (non-symbiotic nitrogen fixing organisms) especially Azotobacter and Azospirillum has improved the yield of annual and perennial grasses (Tilak et al., 2005), just as cyanobacteria nitrogen fixation is essential in the cultivation of rice. Azotobacter also encourage high yield of wheat by over 30% (Gholami et al., 2009). The initiation of molecular dialogue between host plants and soil bacterial occurs through the release of signal in the form of communication chemicals such as flavonoid (Fig. 1) (Perret et al., 2000; Spaink, 2000). This molecule enhances plants-microbe relationship. Barriuso et al. (2008) observed that this chemical aid in the selection of most compactable partners for their growth and subsequent elimination of suspected harmful once. The communication signal is perceived by a bacteria receptor (NodD) and acts as a transcriptional activator of other nodulation genes (nodA, nodB, nodC and nodF) (Franche et al., 2009). The Nod factors activate agent of root nodules residence in the rhizobia (Long, 2001).

Solubilization of Phosphate

Phosphate is next to nitrogen in the list of essential minerals mostly required by plants. However, their deficiency in soil limits crop growth (Nisha et al., 2014). It’s an insoluble inorganic element which increases the economic viability of any agricultural product when solubilized. The organic forms are found mostly in humus and decayed organic materials. Phosphate represent about 0.2% of plants dry weight as it is essential constituent of nucleic acid, phytin and phospholipid. Moreover, its plays a key role in photosynthesis, respiration, storage and transfer of energy during cell division and elongation (Sagervanshi et al., 2012). A large portion of soluble inorganic phosphate is applied to the soil as fertilizer. Due to its rapid rate of fixation and complex formation with other soil elements, it is speedily immobilized and become unavailable to plants (Chatzipavlidis et al., 2013; Vikram and Hamzehzarghani, 2008). Organic materials constitute an important reservoir of immobilized phosphate, accounting for about 20-80% of total soil phosphorus.
A greater proportion of insoluble inorganic phosphate (apatite) or insoluble organic phosphates (inositol phosphate, phosphomonesters and phosphotriesters) are inaccessible by plant (Khan et al., 2007; Chatzipavlidis et al., 2013; Pérez-Montano et al., 2014).

Microorganisms have been identified to play an important role in availing phosphorus to plants through their participation in soil phosphorus cycle. These organisms (PGPRs) directly solubilize and mineralize inorganic phosphorus and facilitate the mobility of the organic forms through biogeochemical cycle (Richardson and Simpson, 2011). Specifically, Phosphate Solubilizing Bacteria (PSB) such as Arthrobacter, Pseudomonas, Alcaligenes, Bacillus, Burkholderia, Serratia, Enterobacter, Acinetobacter, Azospirillum, Azotobacter, Flavobacterium, Rhizobium and Erwinia (Zaidi et al., 2009) have been implicated. Explicitly, each genus act independently to facilitate the dissolution and uptake of phosphate via In vitro condition (Ramachandran et al., 2007). The PSBs secrete organic acids e.g., carboxylic acid, formic acid, propionic acid, lactic acid, glycolytic acid, succinic and fumaric acid (Vazquez et al., 2000). Kaur et al. (2016) in their discovery established that these organic acids lowers the pH in the rhizosphere, thus causing release of the bound forms of phosphate like Ca₃(PO₄)₂ in the calcareous soils. Apart from creating the availability of accumulated phosphate, phosphorus biofertilization also help in increasing the efficiency of biological nitrogen fixation and the availability of Fe, Zn, etc., through production of plant growth promoting substances. PSB are also able to mineralize the insoluble organic phosphate through the excretion of extracellular enzymes such as phytases and C-P lyases phosphatases (Weyens et al., 2010). Authors have reported increase yield of maize (Zea maize) (Yazdani et al., 2009), alfalfa (Medicago sativa L.) (Rodriguez and Fraga, 1999) and soybean (Glycin max) (Abd-Alla, 2001), through PSB inoculation when applied singly or in combination of other rhizobacteria (Mahdi et al., 2010; Ahemad and Khan, 2011).

**Biostimulation**

These are organic chemical compounds that influence plant growth. They are also known as plant growth regulator or phytostimulant e.g.; Auxin (indole-3-acetic acid (IAA)), Gibberellic acid (GA), cytokinins and ethylene. These chemical molecules are recognized over the years as four major plant hormones needed for biochemical and physiological development. PGPR species belonging to the genera Bradyrhizobium, Acetobacter, Azospirillum, Xanthomonas, Alcaligenes, Azospirillum, Enterobacter, Pseudomonas and Klebsiella and also the species of Bacillus pumilus, B. licheniformis, Paenibacillus polymyxa, Phosphobacteria sp, Glucanoacetobacter sp, Aspergillus sp and Penicillium niger has the ability of producing phytohormones (Shobha and Kumudini, 2012; Chatzipavlidis et al., 2013).

**Biostimulation Mode of Actions**

These are PGPR phytostimulators also called plant growth regulator. They are plants exogenously synthesized hormones that regulate plants growth and developments. Its chemical structure is similar to that of natural plant hormones. The mechanism that is being projected is the production of phytohormones (plant...
hormones) such as auxins, cytokinins and GA (Somers et al., 2004). As an organic substance found in extremely low amounts that exert influence on the biochemical, physiological and morphological processes in plants; their production is efficiently regulated. IAA enhances plant nutrition and development, extensive differentiation and increasing rate of xylene and root development (Glick, 2012). Essentially, ethylene is metabolite for normal growth and development, while cytokinin exercises its strength in plant root and shoots cell division (cytokinesis) (Khalid et al., 2006).

**Indole-3-Acetic Acid (Auxin)**

Auxin is an essential molecule that regulates directly or indirectly most plants processes. Being the first phytohormone discovered by Darwin (1880) using *Phalaris canariensis* seeds, it has since paved way for more discovery leading to identification of Indole-3-Acetic Acid (IAA) as the most active and famous plant hormones of auxin group. Irrespective of plants being able to synthesize this chemical molecule (endogenously), they still depend largely on external supply (exogenous) for their optimum performance. This exterior demand is predominantly run by PGPR and associate soil bacterial (Patten and Glick, 2002; Khalid et al., 2006).

Auxin function promptly through the formation of a number of cellular functions e.g., delineation of vascular tissues, initiation of lateral and adventitious roots, stimulation of cell division, elongation of stems and roots and orientation of root and shoot growth in response to light and gravity (Glick, 1995). For PGPR to produce IAA efficiently, the type of specie and strain, its culture condition, developmental stage and availability of nutrient in the rhizosphere are of important (Ashrafuzzaman et al., 2009). Although other auxins, such as indole-3-butyric acid (IBA) and phenyl acetic acid (PAA) have also been identified in plants (Normanly, 1997), their complexity and mode of actions are yet to be understood. Contrary, Bacteria IAA Producers (BIPs) are found to be most abundant in the soil/plant auxin pool and L-Tryptophan (L-TRP) as a precursor that aid increase and production of auxin. This was demonstrated in *B. amyloliquefaciens* FZB42 (Idris et al., 2007), *Fluorescent Pseudomonas* (Karnwal, 2009) and *Azotobacter* and *Azospirillum* strains in canola plant (Yasari and Patwardhan, 2007). Bartel (1997), proposed that rising level of L-Tryptophan increases the biochemical and metabolic activities of BIPs or Auxin Producing Bacterial (APBs), with a corresponding response in root length and modifications of root architecture. The four main metabolic pathways dependent of tryptophan are: tryptophol, tryptamine, indole-3-pyruvic acid and indole-3-acetamide pathway. Emerging evidence illustrate that organisms which produce low quantity of auxin as a result of absence of L-Tryptophan have the propensity of turning up high amount when augmented with L-tryptophan, especially in the presence of viable strain of *Rhizobium* (Zahir et al., 2010). It’s interesting to note that even though the indigenous auxin (IAA) contribute to plant growth, its might still not be adequate for optimum growth performance. Hence, justifying the exogenous need of the chemical messenger (IAA produced by PGPR) to bring to the peak; plant growth, development and adaption to stressed environment.

**Gibberellic Acid Synthesis**

The exact mechanisms by which PGPR promote plants growth via the synthesis of gibberellic acid are not yet understood. It has been known that GA support the development of stem tissue, root elongation and lateral root extension (Yaxley et al., 2001). GA constitute a group of tetracyclic diterpenes that greatly influence the processes of seed germination, leaf expansion, stem elongation, fruit development, flower and trichome initiation (Yamaguchi, 2008). Because of their vital role in improving efficient photosynthetic processes in plants, gibberellins and its producing genera remains the primary target during environmental stress condition, making it an important plant growth bioregulator that can increase the stress tolerance of many crop plants. The improvement of plant growth by some rhizobacteria (PGPR) producing gibberellins was reported (Kang et al., 2009). The exogenous application of these growth hormones may be useful in amendment of polluted soil and improvement of crop performance (Iqbal et al., 2011). Application of GA has shown to increase considerably the grain yield in wheat (Iqbal et al., 2011), barley (Vettakkorumakankav et al., 1999) and tomato by decreasing stomatal resistance and improved water use efficiency (Maggio et al., 2010). Conclusively, gibberellin is involved in plant biochemical modification and stimulates the development of aerial part (Van Loon, 2007) as they remain an excellent alternative for inducing stress tolerant.

**Cytokinin Production**

Cytokinin play a significant role in cell division, vascular differentiation nutrient mobilization, chloroplast biogenesis, shoot differentiation, leaf senescence, apical dominance, anthocyanin production and photomorphogenic development (Davies, 2004). It participates also in vascular cambium sensitivity, proliferation of root hairs and contrarily in inhibition of lateral root formation and primary root elongation (Aloni et al., 2006). This molecule can be acquired endogenously and exogenously by either plant or PGPR respectively. Plants increase uptake of endogenous cytokinin via the promotion of biosynthesis (Pospíšilová, 2003b). Studies have shown that during plant growth, cytokinin perfectly regulates plant adaptation especially in salt polluted soil (Hadiarto and Tran, 2011). Biochemical studies have revealed that cytokinin serve as a major antagonist to
Abscisic Acid (ABA), thus resulting in metabolic alteration of other phytohormones (Pospíšilová, 2003a). During water scarcity, plant cytokinin content reduces drastically with a resultant positive increase in ABA concentration. Assessing the production of plants hormones by different Streptomyces strains, in broth medium shows that all strains synthesized cytokinins and gibberellins (Mansour et al., 1994). Though this is vital for phyto development, its receptor gene in plants is often regulated by changes in osmotic conditions (Merchan et al., 2007).

Ethylene

Ethylene is a unique phytohormone with wide range of biological activities. The beneficial role of this biomolecule is best recorded at low concentration. Ethylene hinders some key developmental properties e.g., root elongation, induce defoliation and other cellular processes at high concentration resulting to reduced crop performance (Bhattacharyya and Jha, 2012). Pierik et al. (2006) was of the opinion that ethylene classification as a senescence hormone was due to its inhibitory role to plant growth. To overcome these alarming consequences, an enzyme 1-Aminocyclopropane-1 Carboxylic acid (ACC) deaminase is needed. The role of this biocatalyst is to degrade the plant ACC which is the direct precursor of ethylene synthesis in plant to α- ketobutyrate and ammonium (Glick et al., 2007). The result of the degradation is the reduction of plant ethylene production through a range of mechanisms, while the PGPR producing ACC-deaminase regulates the ethylene level in plant and prevents the growth inhibition caused by high levels of ethylene (Noumavo et al., 2016). PGPR capable of inducing exogenous production of ethylene via degradation of the endogenous product using enzyme include Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia and Rhizobium. Works has shown that PGPR ACC deaminase activities were vital for Brassica napus growth (Dell’Amico et al., 2008). Pierik et al. (2006) suggested that at low concentration of ethylene mediated by PGPR, the plant yield, growth performance and germination properties of Arabidopsis thaliana get accelerated. However, this vapidous hormone regulate also root initiation, fruit ripening, seed germination, leaf abscission and wilting (Kaur et al., 2016).

PGPR in Phytoremediation

As soil constantly welcome large influx of waste materials, its overtime exert stern impact on the environment and human health. Most common of these pollutants are heavy metals (Hg, Pb, Cr, Co, Zn, Ni and Cd). These metals have also been attributed to industrialization, urbanization and civilization (Odoh et al., 2017a). In agricultural practice, this form of pollution has been traced to human activities such as excessive fertilizer application, indiscriminate disposal of sewage and municipal waste and pesticides/insecticide usage. Though at immediate, these agro chemicals facilitate growth and productivity and subsequently leave records of metal residues that impair on plant growth and microbial metabolism at a long run. Because they are non-biodegradable, their remediation process becomes extremely difficult, and can only be transform from one state to another. Soil rhizobacteria assisted phytoremediation has become an alternative of choice in detoxifying sites because it’s cost effective, ecofriendly and aesthetic (Odoh et al., 2017b). Decontaminating these heavy metals polluted land occur through chelation, solubilization and mineralization using large consortium of soil microorganisms. These however aid their bioavailability/mobility and bioaccumulation during phytoremediation.

Commercialization of PGPR and its Challenges in Africa

Despite the knowledge gap of PGPR by agriculturists in the developing and less developed world, a good number of bacteria have long been used (Banerjee et al., 2006) for agro practices in advance countries and emerging economy (Table 2).

Although PGPR benefit is so enormous, its implementation is still at a developmental stage considering the increasing world population and demand for agricultural product. Effective and efficient utilization of this biotechnology for aggressive food production in the wake of rising food scarcity and humanitarian need is paramount. More In-situ research base approach should be carried out to ascertain the most suitable strain and appropriate biotic condition needed for growth, while paying good attention on the soil quality/property and season of their optimum performance.

There is need for government agencies in the tropics to enact policy and regulation regarding strain of organisms to be released into the environment (Glick, 2012) and also clarify their stake on genetically modified organisms as it will increase yield and turn-up in agrobiology.

More works need to be done in the tropics to commercialize agriculture i.e. (industrial agriculture) that has for decades been left in the hands of peasant farmers who are ill equipped with modern obtainable practice. With the dwindling economy as a result of fall in oil price that has affected most mono-economy nation such as Nigeria. Agriculture still remain a lifelong viable revenue for the government, as more research need to be done to ascertain PGPRs strain-crop specificity and indebt soil analysis while considering African climatic condition.

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Table 2: List of countries with commercialized bio-product formulated with PGPRs

| Countries     | Manufacturer            | Product               | Organisms                          | Crop                                    |
|---------------|-------------------------|-----------------------|------------------------------------|-----------------------------------------|
| Australia     | Mapleton AgriBiotec pty Ltd | Twin N ®              | Azoarcus indigen NAB 04            | Sugar cane, Vegetables, Cereals, Rape, Sugar beet |
|               |                         |                       | Azospirillum brasilense NAB 317     |                                         |
|               |                         |                       | Azorhizobium caulinodii NAB 38     |                                         |
| Brazil        | Embrafos Ltda           | Bioativo ®            | PGPR consortia, organic matters    | Bean, maize, sugarcane, rice, carrot, cotton |
| Canada        | Lallen and plant care BASF Inc. | Rhizocell ® GC       | B. amyloliquefaciens IT 45         | Cereals and horticultural plants         |
|               |                         |                       | Nodulator®                         |                                         |
|               |                         |                       | Bradyrhizobium japonicum           |                                         |
| China         | China Bio-Fertilizer AG (CBF) |                | PGPR consortia                      |                                         |
| Cuba          | Labfalum S.A            | Nitrofix ®            | Azospirillum sp                     | Wheat, barley, carrot, maize, cabbage    |
| France        | Biovitis                | Ceres ®               | P. fluorescens                     | Horticultural crop                      |
|               |                         |                       | Streptomyces griseoviridis         | Ornamentals, Tree seedlings             |
| Finland       | Kemira Agro Oy          | Mycostop ®            |                                    |                                         |
| Germany       | AhiTEP GmbH             | EZB 24 ® fl           | B. amyloliquefaciens sp. plantarium| Vegetables                             |
|               | Agro bio Hungary kft    | Bactofila10®          | A. Brasile. A. Vinel.              |                                         |
| Ireland       | Biomax                  | Greenmax              | PGPR consortia                      | wide range of plant                     |
|               |                         | Life®, BioMix®, Biaziwk®, Biodine® |                         |                                         |
|               |                         | GMx PGPR              |                                    |                                         |
| Italy         | CCS Aosta Srl           | Micosat FR® cereali   | B. subtilis BR62, Peantibacillus    | Tomato, soybean, cereals, beet, sunflower |
|               |                         |                       | dura PD74, Streptomyces sp ST60     | Legumes                                |
| Japan         | Tokachi Federation of Agricultural Cooperatives (TFAC) | Mammezo®, R- Processing Seeds®, Hyper Coating Seeds ® | Rhizobium based formulation in peat, legume seeds and grass legume seed. | |
| New Zealand   | Borty-Zen 2010 Ltd      | Armor-Zen®            | Chitosan. An elicitor against Botrytis cinerea (grey mould), Sclerotinia schroeforum (white rot) | Grapevine, ornamentals |
| Spain         | LAB (Labtech)           | Inomix ® biostimulant | B. polymyxa (LAB/BP/01), B sulitus (LAB/BS/F1) | Cereals |
| United Kingdom (UK) | Cleveland biotech | Aminite A 1000® | Azotobacter, Bacillus, Rhizobium, Cheetanion, Pseudomonas | Cucumber, lettuce, tomato, peper |
| United States of America | AgBioChem | Galtrol ®          | Ageobacterium radiobacter strain 84 | Ornamentals, Fruits, Nuts |
|               | Plant Health Care       | Complete ® plus       | B. pumilus, B. subtilis, B. lichenformis, Trichoderma harzianum T-39 | Nursery trees and field crop |
|               | Bio works               | Trichodex ®           | B. polymyxa, B. azotofixan, B. megaterium | Food crops |

There is need for proper campaign and education of local populace on the important of genetically modified organisms and microbial inoculants in agro base practice, so as to explore the full potential of her rich fertile land for sustainable food production.

Conclusion

Decades ago, for an agricultural practice to be successful one must not neglect the use of chemical fertilizer, herbicides and pesticides. At an initial point, these chemicals aid plant growth and later exert their negative effects. This norm has not only affected the soil and its inhabitant but also renders threat to human life through the food chain. With rise in soil pollution, change in climatic condition, soil-born pathogen and extensive land overuse, the soil has become grossly infertile and unproductive; this is evident in the low agricultural output, food insecurity amidst the rising human population. To achieve self-sufficiency, effort must be made especially in the tropics to key into scientific knowledge through broad understanding of soil-plant-microbial interaction and their mechanism of action; this will not only lead to bumper harvest but keep the soil safe and healthy. As campaign for the use of PGPR gets heightened, attention should be focused on substituting agrochemicals with bioproduct formulated with consortium of beneficial PGPR. Highlighted advantages of these products in terms of; increased plant nutrient and biocontrol through induction of systemic resistance and nutrients and/or space competition must be carefully stated and comprehended by farmers for enhanced crop yield. Concussively, genetic engineering of PGPR as an integral constituent in modern food production will mitigate soil pollution, ecosystem alteration and destruction of soil flora and fauna when properly harnessed especially in developing economy.

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Author’s Contributions

Odoh Chuks Kenneth: He is the lead author and responsible for the drafting of the manuscript, defining concepts and reviewed literatures.

Eze Chibuzor Nwadibe: Supervised and approved the final manuscript.

Akpi Uchenna Kalu: Helped in coordination and editing of the manuscript.

Unah Victor Unah: Controlling abstract as well as in adjusting the paper template.

Ethics

The article is original and the authors have declared no conflict of interest.

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