

**COMPARATIVE GROWTH RESPONSES OF *Amaranthus* spp.  
L. IN HUMUS AND FERRUGENOUS ULTISOLS USING  
PLANT GROWTH PROMOTING RHIZOBACTERIA**

**BY**

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**DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY  
FACULTY OF LIFE SCIENCES  
UNIVERSITY OF BENIN**

**DECEMBER, 2019.**

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF  
PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE  
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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE  
AWARD OF MASTER OF SCIENCE (M.Sc. HONS) DEGREE IN  
PLANT BIOLOGY AND BIOTECHNOLOGY**

**DECEMBER, 2019.**

## CERTIFICATION

This is to certify that this project was prepared and presented by Funmilayo Oluwaseun FAWEHINMI (Miss) of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin-city, Edo state, Nigeria.

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## **DEDICATION**

This work is dedicated to the Almighty GOD

## ACKNOWLEDGMENT

My appreciation goes to The Almighty God,

I am indeed very grateful to my supervisor, Dr. B. Ikhajiagbe for his fatherly support and commitment to making me a better scientist. My appreciation also goes to Prof. G. O. Anoliefo for all his kindly support and encouragement throughout the study.

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## ABSTRACT

The study was conducted to investigate the possibility that growth-promoting rhizobacteria would enhance plant growth capacities of *Amaranthus hybridus* and *A. viridis* in ferruginous ultisols, comparative to their humus counterparts; given the negative impact the former has on cultivation of the plant species. To achieve this, the setup was divided into two groups; the first comprised of 2 weeks old plants that were inoculated the test rhizobacteria – *Pseudomonas putida*, *P. fluorescence* and a combination of both in the ratio of 1:1, in both humus (HMS) and ferruginous red (FRS) soils. The second consisted of seeds of the test plants bioprimered with the bacteria in filtrates of HMS and FRS respectively. Growth responses were observed and measured. Plant species sown in the ferruginous red soils (FRS) did not survive beyond 45 days notwithstanding the kind of microbial inoculant. They also did not attain a plant height of 3cm and as such were not reasonable for harvest. For plants sown in humus soil (HMS), height ranged from 30.1 – 39.2 cm in *A. hybridus* ( $p=0.293$ ) and 35.3 – 41.6 cm ( $p=0.072$ ) in *A. viridis*, notwithstanding the rhizo-inoculant used. For *A. hybridus*, Leaf area was significantly higher in the inoculated plants sown in HMS. No significant changes in leaf area was reported for *A. viridis* irrespective of exposure to rhizo-inoculation (11.5 – 17.5 cm<sup>2</sup>,  $p=223$ ). No significant differences in number of leaves per plant, stem girth, and internode was reported in both species irrespective of treatment. Qualitative assessment of phytochemicals showed presence of saponins, phenolic, tannins, terpenoids, alkaloids, and flavonoids in both species notwithstanding inoculation. The presence of glycosides in plants inoculated with *P. pseudomonas* was reported. Glycosides were absent in the control plants. Significant reduction in total phenols upon inoculation of plants with *P. putida* was also reported. Following the failure of FRS-exposed plants to subsist, in-vitro growth studies were conducted to compare seed growth response. Results showed that seeds sown in FRS-filtrates grew as better as those in HMS-filtrates. Germination percentage in the *Pseudomonas*-exposed seeds was better when seeds were primed for 24h than ofr 3 h in both plant species.

## CHAPTER ONE

### 1.0

### INTRODUCTION

#### 1.1 Background of Study

Vegetables are occupying a coveted position in Nigeria agriculture and the area under vegetables is accounted as 8.49 Mha area with annual production of 146.55 Mt (Vasquez *et al.*, 2011). Vegetable environment is severely threatened by abiotic and biotic factors. Among which crop loss due to biotic factors especially due diseases is huge and estimated as 50-80 % from the heavily infected fields. Farmers are extremely concerned for their crop, and for a quick remedy they use excessive amount of chemicals to control the diseases which in turn leads to ill effects on environment and no targeted organisms including animals and human beings (Verma and Poonia, 2003). To maintain an ecological balance, protection measures need to be resorted to which are based on less or zero use of chemicals. Alternatively, use of microbes is shown to be an attractive method because in this method, a beneficial interaction or mutual relationship between the host plant and microorganisms can be achieved PGPR are group of bacteria that generally colonize in the rhizospheric zone of the plant tissue, encourage plant growth in different ways and control various plant diseases (Steiger *et al.*, 2014) and some of the best examples of these bacterial genera are *Bacillus*, *Pseudomonas* and *Bradyrhizobium*. PGPR facilitate to enhance plant growth through various activities like chelation of iron (siderophore), fixation of nitrogen, production of phytohormones and solubilization of insoluble phosphorus (P).

*Amaranthus* species is readily available tropical vegetables found in many countries located across the equator region. *A. hybridus* and *A. viridis* shared similar ecological vegetable properties as they are commonly grown edible plants (Sahrawat, 2000). *A. hybridus* and *A.*

*viridis* as a vegetables contain high amounts of vitamins and minerals such as phosphorus, magnesium, calcium, potassium and others which are required in our diet for a healthy living (Ahmad *et al.*, 2007) and are often regarded as the daily staple diet for many people. Vegetables are able to provide energy as it consists most of the essential nutrients, such as proteins, carbohydrates, minerals, vitamins and other trace elements. Even though vegetables are an important component of our daily diet, there is little information available as to its contamination by heavy metals in ferruginous soil. A common example of contamination includes bioaccumulation of heavy metals in vegetables (Assuncao *et al.*, 2003). Bioaccumulation refers to the increase in concentration of a particular chemical or element in biological organisms over time and can pose a threat to the well-being of plants, animals and human beings. It is well documented that heavy metals inhibit many enzymes and thus able to disrupt metabolic processes, including photosynthesis in plants. Most people assume that all vegetables are nutritious as well as safe to consume, unaware that some parts of the vegetable may be contaminated with heavy metals and other sources of contaminants (Barcelo and Poschenrieder, 2004).

Heavy metals are non-biodegradable and can be very persistent in the environment; have the potential to accumulate in different body organs. By consuming contaminated vegetables, excessive accumulation of dietary heavy metals such as cadmium, lead and chromium can lead to severe health problems in humans. Heavy metals contamination in food specifically in vegetables, have been reported in many countries including Nigeria and other African countries. Cempel and Nikel (2006) revealed that heavy metals have different effects on various vegetable plants. Plants have the ability to accumulate metals from the environment and can be categorized as unsafe for consumption if the plants are cultivated on or near to contaminated land. Plants require many sorts of essential macro and micro (trace) mineral nutrients for normal growth and development and these include nitrogen, phosphorus,

potassium, magnesium, calcium, iron, zinc and sulphur. However, vegetable plants such as okra can easily absorb and take in heavy metals naturally into their vacuoles (Chen *et al.*, 2009). It has been reported that both lead and cadmium are the most common heavy metals in soils while all other types of heavy metals are significantly toxic in high concentration amounts. The ingestion of vegetables grown in such contaminated soils will pose a danger to both animal and human health.

## **1.2 Justification of study**

Plants have been faced with various adverse environmental conditions. These conditions result in various metabolic disorders in plants, this state of the plant is referred to as stress condition and have been the major cause of agricultural yield lost. Such environmental factors causing stress can be divided into two namely, abiotic and biotic in relation to whether it is caused by a living thing or not. It therefore implies, that stress could be as a result of either abiotic or biotic factor. Stress of abiotic origin are referred to as abiotic stress and they include water stress or drought, temperature stress (heat and cold stress), pH stress, others includes stresses imposed by the various soil nutrients such as iron, calcium, phosphorus, potassium, among others. Stress of the biotic origin is referred to as biotic stress and they can include infestation by different kinds of phytopathogens such as bacteria and fungi, nematodes, grazing by herbivores, and cutting by human among others.

However, because the plants always stay in the soil, most of the stresses that come their way are relatively soil related, except I cases relating to light condition. The reason by which plants on the same piece of land will usually experience the same type of stress. Prominent among the soil related stress is water stress which deals with the availability of water in the soil to the plant, salinity stress which is as a result of increase in the solute concentration of the soil to an extent that is higher than that of the root system of the plant, and nutrient stress.

Nutrient stress is usually in two phases, it is either the deficiency of the nutrient required for the proper growth of the plant leading to an abnormality in the growth of the plant or otherwise called disease symptoms and could lead to a reduction in the plants yield. A typical example is what happens when nitrogen is deficient in the soil, the plant in this condition shows symptoms such as chlorosis, retarded growth and definitely a reduction in the yield of such crop. Another case is when iron is limited in the soil, the plant suffers great metabolic irregularities because of the involvement of iron in major metabolic process such as in photosynthesis, respiration and important role in enzyme function.

However, the plant can be faced with the other phase of nutrient stress which occurs when one or more nutrient element is in higher concentration in the soil than it is required by the plant up to a level that it becomes toxic to the plant. This is especially of great importance when the overly abundance of this element starts affecting the uptake of other element such as in the case of excess sodium when the amount of salt is in abundant, start affecting the uptake of potassium ion, over accumulation in the plant up to a toxic level. This will tell negatively on the growth pattern of the plant and could ultimately result to the wilting in such plant. Sometimes, micronutrient could also could also reach a level in the soil even above the level of the major minerals in the soil as in the case of the red soil which characteristically red because of the overly abundance of iron in the soil.

This leads iron stress on the plant. The plants in such cases are said to accumulate excess iron into their system up to toxic level, the toxicity could either results in the inability of the plant to absorb other nutrients such as manganese, potassium, phosphorus, calcium and magnesium or leads to oxidative stress in the plant as the toxicity leads to the production of hydroxyl radicals (OH<sup>-</sup>) and reactive oxygen species (ROS) because of the involvement of iron in Fenton reaction, thereby cause damage to the DNA, proteins, membrane lipid and subsequently damage to the chlorophyll and as a result reducing photosynthesis. Iron stress

will result in the death of the plant if the condition is not alleviated in the soonest possible time.

The interaction between plant and bacteria have been a major project for scientist, as they have been found to be beneficial to plant at some point in contrast to the general believe that they are phytopathogens. These group of bacteria that have been found to be beneficial to plants in that they promote growth, increase yield, and serve as biocontrol agent against pest and pathogen. These bacteria are called plant growth promoting rhizobacteria (PGPR). They use various mechanisms to promote the growth of plants. The various mechanism adopted by various bacteria is solely dependent on their physiological capabilities such as the ability to produce siderophore which not all bacteria can produce.

However, the mode of growth promotion is generally divided into direct and indirect mechanisms. Because one of the major reasons for reduced yield of plant produce is the fact that they are faced with different forms of stress, work have been done to show that PGPRs have the ability to alleviate some level of plant stress and thereby promoting growth. The mechanisms by which PGPRs do this is by one of the following reasons; production of phytohormone such as auxin, gibberellin, cytokinin, ethylene and abscisic acid, the production of antibiotics against plant pathogens such as pyrrolnitrin, phenazine, pyoluteorin, cyclic lipopeptide , phloroglucinol and hydrogen cyanide, making available plant required nutrients either through fixation as in the case of nitrogen, solubilization in the case of phosphorus, or through the use of siderophores which is specialized in harvesting the iron content of the soil especially in cases of deficiency. Other mechanisms involved in the promotion of growth is through both acquired systemic resistance and induced systemic resistance.

In the case of water stress, which results when the water content of the soil is not readily available either due to draught or because of increase in the solute concentration of the soil such that it becomes hypertonic as against the concentration of the root. PGPRs have been reported to help by the production of hormone which would enhance root proliferation which could help the plant in better search of water. This is also applicable in the case of nutrient deficiencies. Apart from root proliferation, nutrients such as nitrogen and phosphorus can be made available as described earlier. However, the most prominent method of stress alleviation is through the production of volatile organic compounds (VOCs). These compounds have been reported to be responsible for the activation of certain genes which are responsible for stress tolerance in plants.

This is the case for salinity stress in which the concentration of Na ion and chloride ion is in excess in the soil. Liu and Zhang (2015) reported the case of Arabidopsis plant under salt stress when treated with a PGPR strain called GB03 VOCs showed greater biomass production and less Na<sup>+</sup> accumulation when compare to salt-stressed plants without VOC treatment (Zhang et al., 2008a). The VOC-induced stress tolerance was attributed to the activation of the HKT1 gene of the Arabidopsis plant. HKT1 is a xylem parenchyma-expressed Na<sup>+</sup> transporter that is responsible for Na<sup>+</sup> exclusion from leaves by removing Na<sup>+</sup> from the xylem sap (Sunarpi et al., 2005; Horie et al., 2009; Møller et al., 2009). Under salinity stress, GB03 VOCs reduce Na<sup>+</sup> accumulation in Arabidopsis shoots, presumably by enhancing HKT1-dependent shoot-to-root Na<sup>+</sup> recirculation, because VOCs transcriptionally up-regulate HKT1 in shoots and concomitantly down-regulate HKT1 in roots (Zhang et al., 2008a).

This means that the bacteria have the ability to enhance growth by regulating the transcription of certain beneficial genes in the plant necessary for stress tolerance. In the case of iron stress, Mahender et al. 2019 in a review reported that one of the effect of iron toxicity is by

hampering the ability of the plant to take up other nutrients such as calcium, potassium, manganese and phosphorus, it is therefore report that PGPR can alleviate the effect of iron stress by making available to the plant and enhancing absorption of the nutrient. A major example is the solubilization process that the PGPR are known for, the siderophores of PGPR have also been reported to be able to pick up other metals not just siderophore. In fact, siderophores only pick up iron in times when iron is limited in the soil. It has been reported that plants that are susceptible to iron stress lacks a functional GSNOR gene, because PGPRs have the ability to regulate the stress related genes in plant through the production of VOCs, therefore the regulation of the GSNOR gene by the VOC of PGPRs could also be one of the mechanisms used by plants to induce iron tolerance in plant facing iron tolerance.

*Pseudomonas* is a genus of the gram-negative bacteria which have been shown to possess various attribute to promote the growth of plant. They are one of the major constituents of the bacteria that constitute the plant growth promoting rhizobacteria (PGPRs). Most of them have been isolated in at the rhizosphere, some have also been isolated as endophyte in some plant roots. They have been reported in research works even many more are still on going on their incorporation in agriculture and have proven to serve as a better alternative to the conventional agricultural practices which uses chemicals fertilizers to enhance plant yield but at the long run has an ugly effect on the environment. In an attempt to disdain the use of chemical fertilizers, agriculturist has shifted focus to practices which is aim at been environmentally friendly and at the same time cost effective.

One of the major practices that have received a thumb up is the use of organic fertilizers, however the use of microbes has also been embraced. One of the major microbes that have gained attention is the genus *Pseudomonas*. Among this genus, the species of *P. aeruginosa*, *P. fluorescens*, *P. putida* have been extensively worked on and have been reported to promote the growth of plants. They have been reported to increase yield and act as biological control

of pest and pathogens. Ramette et al. (2006) reported that *Pseudomonas* are plant growth promoting bacteria, because they have shown high ability of IAA production, phosphate solubilization and siderophore production. Therefore, they have been incorporated into use as substitute for especially chemical fertilizers to increase the yield of various type of crops.

In an experiment conducted by Adesemoye and Ugoji (2009) on *Abelmoschus esculentus* L. (okra), 57 L. (tomato), and *Amaranthus spp.* (African spinach), using *Pseudomonas aeruginosa* as the PGPR shows that the effect of PGPR is equivalent to that observed when chemical fertilizers were used. However, some of the result of the experiment also showed that the use of PGPR was more productive for the cultivation of *Amaranthus spp.* And that even in other plants the seeds inoculated with the bacteria produced fruit earlier than that of chemical fertilizer. He also reported that the pattern of growth mediated by bacterization through soaking was similar to those mediated by bacterization through coating. Yadav et al. (2010) reported that the growth of chickpea was enhanced due proper root colonization of plant growth promoting rhizobacteria which provide plant hormones (IAA), phosphorus and ammonia to plant and also by the solubilization of precipitated phosphates and enhance phosphate availability to chickpea that represent a possible mechanism of plant growth promotion under field condition.

Sadaghiani *et al.* (2008), demonstrated that the production of siderophore by *pseudomonas spp.* enhance plant growth by enhancing the availability of iron to the plant. The experiment was done by inoculating the plant with a mutant strain which lacks the ability to produce siderophore called MPFM1 and a wild type which have the ability to produce siderophore called 7NSK2. Seeds inoculated with 7NSK2 showed lower degree of iron chlorosis, which was visualized as interveinal yellowing of wheat leaves. In 7NSK2 treatment along with control plant, treatment with 7NSK2 was effective in reducing chlorosis as evident in

increased chlorophyll components, 7NSK2 resulted in a significant dry weight increase in maize compared to MPFM1 strain.

The use of various *Pseudomonas spp.* on various crops have been extensively reported in a review by Singh (2013) where he stated the use of the bacteria species as a biofertilizer. *Pseudomonas putida* when used as biofertilizer enhances early developments of canola seedlings and the growth stimulation of tomato plant, *P. fluorescens* increased the growth of pearl millet, cause increase in growth, leaf nutrient contents and yield of banana (*Musa sapientum*), *P. putida*, *P. fluorescens* also improves seed germination and growth parameters of *P. putida*, maize seedling in greenhouse and also grain yield of field grown maize. *Pseudomonas fluorescens* enhance disease resistance to Sheath blight disease and leaf folder insect in rice (*Oryza sativa*), it reduces the Banana Bunchy Top Virus (BBTV) incidence and saline resistance in groundnut (*Arachis hypogea*).

In a comparative study using different strains of plant growth promoting *Pseudomonas* which include *P. aeruginosa* PW-99, *P. aeruginosa* PW-136, *P. putida* PW-2, *P. putida* PW-56, *P. cepacia* PW-18, *P. cepacia* PW-43, *P. fluorescens* PW-5 and *P. fluorescens* PW-104. All the *Pseudomonas* strains enhanced plant growth in rice plant. However, *P. fluorescens* PW-5 produced maximum shoot, root and dry weight of rice plant by 157.72, 408.06 and 233.84% respectively as compared to *P. aeruginosa* PW-99 increased shoot, root and dry weight of rice plant by 154.70, 237.63 and 210.26 %, *P. aeruginosa* PW-136 enhanced shoot, root and dry weight of rice plant by 151.47, 231.18, and 199.61 %, *P. putida* PW-2 improved shoot, root and dry weight of rice plant by 52.80, 172.04, and 93.15 %, *P. putida* PW-56 enhanced shoot, root and dry weight of rice plant by 67.69, 165.59, and 100.38 %, *P. cepacia* PW-18 enhanced shoot, root and dry weight of rice plant by 51.61, 340.32, and 110.64 %, *P. cepacia* PW-43 increased shoot, root and dry weight of plant by 74.01, 355.37, and 138.02 %, *P. fluorescens* PW-104 enhanced shoot, root and dry weight of plant by 149.78, 406.98, and

223.19 %. Deshwal and Kumar, 2013. On the analyses of the plant growth promoting properties of pseudomonads on two wheat cultivars using calcareous soil. The results showed that bacterial strains *Pseudomonas sp.* and *P. fluorescens* were able to colonize the rhizosphere of both wheat cultivars and were able to significantly stimulated the shoot and root length and dry weight of wheat (Egamberdieva, 2010).

### **1.3 Aims of the Study**

This study was carried out to comparatively assess growth response of *Amaranthus* species grown in both humid and red ferruginous ultisoils using plant growth promoting rhizobacteria

Specific objectives of this study are to;

1. comparatively determine the morphological growth parameters of the test plant under experimental conditions
2. quantitatively determine phytochemical compound present in the leaves of the tested plant
3. quantitatively determine the total phenol present in the plant sample
4. determine the germination effect of the tested plant exposed to red ferruginous soil

## CHAPTER TWO

### 2.0

### LITERATURE REVIEW

#### 2.1 Soil

Soil is a major component of the earth's ecosystem. The world's ecosystems are impacted in far-reaching ways by the processes carried out in the soil, from ozone depletion and global warming to rainforest destruction and water pollution. With respect to Earth's carbon cycle, soil is an important carbon reservoir, and it is potentially one of the most reactive to human disturbance and climate change (Khellaf and Zerdaoui, 2010). As the planet warms, it has been predicted that soils will add carbon dioxide to the atmosphere due to increased biological activity at higher temperatures, a positive feedback (amplification). This prediction has, however, been questioned on consideration of more recent knowledge on soil carbon turnover. Soil acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth, making it a critically important provider of ecosystem services (Lin and Kao, 2007). Since soil has a tremendous range of available niches and habitats, it contains most of the Earth's genetic diversity.

A gram of soil can contain billions of organisms, belonging to thousands of species, mostly microbial and largely still unexplored. Soil has a mean prokaryotic density of roughly  $10^8$  organisms per gram, whereas the ocean has no more than  $10^7$  prokaryotic organisms per milliliter of seawater (Pandey and Sharma, 2002). Organic carbon held in soil is eventually returned to the atmosphere through the process of respiration carried out by heterotrophic organisms, but a substantial part is retained in the soil in the form of soil organic matter; tillage usually increases the rate of soil respiration, leading to the depletion of soil organic matter. Since plant roots need oxygen, ventilation is an important characteristic of soil

(Pareek *et al.*, 1990). This ventilation can be accomplished via networks of interconnected soil pores, which also absorb and hold rainwater making it readily available for uptake by plants. Since plants require a nearly continuous supply of water, but most regions receive sporadic rainfall, the water-holding capacity of soils is vital for plant survival.

## **2.2 Red soil**

Red soil is an important soil resource, which bears substantial implication for sustainable development of agriculture and healthy growth of economy. However, the red soil in Edo state has been deteriorating in recent years and facing many threats, such as soil erosion, acidification, and pollution (Slininger *et al.*, 2010). Among these, contamination of heavy metals, particularly arsenic and cadmium pollution in paddy soils of the red soil regions, has become a major environmental concern. The color of red soil ranges from red to brown, chocolate, yellow, gray, or sometimes even black. Red soil contains a high percentage of iron content, which is responsible for its color. This soil is deficient in nitrogen, humus, phosphoric acid, magnesium, and lime but fairly rich in potash, with its pH ranging from neutral to acidic (Singh and Singh, 2001). It is formed by the weathering of ancient crystalline and metamorphic rocks, particularly acid granites and gneisses, quartzitic rocks, and felspathic rocks.

Chemically, red soil is siliceous and aluminous, with free quartz as sand, but is rich in iron, ranging from sand to clay with the majority being loamy. The lowermost area of red soil is dark in color and very fertile, while the upper layer is sandy and porous. Their chemical composition include non-soluble material 90.47%, iron 3.61%, aluminium 2.92%, organic matter 1.01%, magnesium 0.70%, lime 0.56%, carbon dioxide 0.30%, potash 0.24%, soda 0.12%, phosphorus 0.09% and nitrogen 0.08% (Verma and Poonia, 2003). However significant regional differences are observed in the chemical composition.

### 2.3 Rhizosphere

The slender sector of soil directly surrounding the root machine is referred to as rhizosphere, while the time period 'rhizobacteria' implies a set of rhizosphere bacteria ready in colonizing the root environment. Similarly, to imparting the mechanical assist and facilitating water and nutrient uptake, plant roots additionally synthesize, acquire, and secrete a diverse array of compounds (Maheshwari *et al.*, 2012). those compounds secreted by means of plant roots act as chemical attractants for a sizeable number of heterogeneous, numerous and actively metabolizing soil microbial communities. The chemicals which can be secreted by way of roots into the soils are usually known as root exudates. The exudation of an extensive range of chemical substances modifies the chemical and bodily residences of the soil and for that reason, regulates the structure of soil microbial community in the on the spot area of root floor. In reality, some of the exudates act as repellants in opposition to microorganisms even as others act as attractants to motel the microbes (Viveros *et al.*, 2010). The composition of these exudates is dependent upon the physiological popularity and species of vegetation and microorganisms.

Moreover, these exudates additionally sell the plant-useful symbiotic interactions and inhibit the boom of the competing plant species. Additionally, microbial activity within the rhizosphere influences rooting styles and the delivery of available vitamins to flowers, thereby editing the fine and amount of root exudates. a fragment of those plant-derived small organic molecules is similarly metabolized by way of microorganisms within the place as carbon and nitrogen resources, and some microbe-orientated molecules are ultimately re-taken up by using vegetation for increase and improvement (Ahemad and Kibret, 2014). Certainly, carbon fluxes are vital determinants of rhizosphere feature. It's far suggested that about 5–21% of photosynthetically constant carbon is transported to the rhizosphere thru root exudation. As a consequence, the rhizosphere can be described as any quantity of soil in

particular influenced by way of plant roots and/or in affiliation with roots hairs, and plant-produced substances. Largely, three separate however interacting additives are recognized in the rhizosphere: the rhizosphere (soil), the rhizoplane, and the basis itself (Bhattacharyya and Jha, 2012). Of those, the rhizosphere is the area of soil motivated by way of roots thru the release of substrates that affect microbial activity. The rhizoplane, alternatively, is the root floor which include the strongly adhering soil particles even as the basis itself is a component of the device, because many micro-organisms (like endophytes) also colonize the foundation tissues. Microbial colonization of the rhizoplane and/or root tissues is referred to as root colonization, whereas the colonization of the adjoining quantity of soil under the have an effect on of the foundation is called rhizosphere colonization (Kloepper and Schroth, 2011).

## **2.4 Rhizobacteria**

Rhizobacteria are root-colonizing bacteria that form symbiotic relationships with many plants. The name comes from the Greek *rhiza*, meaning root. Though parasitic varieties of rhizobacteria exist, the term usually refers to bacteria that form a relationship beneficial for both parties (mutualism). They are an important group of microorganisms used in biofertilizer. Biofertilization accounts for about 65% of the nitrogen supply to crops worldwide. Rhizobacteria are often referred to as plant growth-promoting rhizobacteria, or PGPRs (He *et al.*, 2010). The term PGPRs was first used by Joseph W. Kloepper in the late 1970s and has become commonly used in scientific literature. PGPRs have different relationships with different species of host plants. The two major classes of relationships are rhizospheric and endophytic. Rhizospheric relationships consist of the PGPRs that colonize the surface of the root, or superficial intercellular spaces of the host plant, often forming root nodules. The dominant species found in the rhizosphere is a microbe from the

genus *Azospirillum*. Endophytic relationships involve the PGPRs residing and growing within the host plant in the apoplastic space (Xiong, 2008).

The symbiotic relationship between rhizobacteria and their host plants is not without costs (Khan *et al.*, 2007). For the plant to be able to benefit from the added available nutrients provided by the rhizobacteria, it needs to provide a place and the proper conditions for the rhizobacteria to live. Creating and maintaining root nodules for rhizobacteria can cost between 12–25% of the plant's total photosynthetic output. Legumes are often able to colonize early successional environments due to the unavailability of nutrients (Ma *et al.*, 2009). Once colonized, though, the rhizobacteria make the soil surrounding the plant more nutrient rich, which in turn can lead to competition with other plants. The symbiotic relationship, in short, can lead to increased competition.

PGPRs increase the availability of nutrients through the solubilization of unavailable forms of nutrients and by the production of siderophores which aids in the facilitating of nutrient transport (Ma *et al.*, 2011). Phosphorus, a limiting nutrient for plant growth, can be plentiful in soil, but is most commonly found in insoluble forms. Organic acids and phosphatases released by rhizobacteria found in plant rhizospheres facilitate the conversion of insoluble forms of phosphorus to plant-available forms such as  $\text{H}_2\text{PO}_4^-$ . PGPR bacteria include *Pseudomonas putida*, *Azospirillum fluorescens*, and *Azospirillum lipoferum* and notable nitrogen-fixing bacteria associated with legumes includes *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, and *Rhizobium* (Wu *et al.*, 2015).

Though microbial inoculants can be beneficial for crops, they are not widely used in industrial agriculture, as large-scale application techniques have yet to become economically viable. A notable exception is the use of rhizobial inoculants for legumes such

as peas. Inoculation with PGPRs ensures efficient nitrogen fixation, and they have been employed in North American agriculture for over 100 years (Nieto *et al.*, 2002).

#### 2.4.1 *Pseudomonas* Species

*Pseudomonas* is a genus of Gram-negative, aerobic Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches. Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research (Rosko and Rachlin, 2007); the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*. Because of their widespread occurrence in water and plant seeds such as dicots, the pseudomonads were observed early in the history of microbiology (Cempel and Nickel, 2006). The generic name *Pseudomonas* created for these organisms was defined in rather vague terms by Walter Migula in 1894 and 1900 as a genus of Gram-negative, rod-shaped and polar-flagellated bacteria with some sporulating species, the latter statement was later proved incorrect and was due to refractive granules of reserve materials (Sharma and Dubey, 2005). Despite the vague description, the type species, *Pseudomonas pyocyanea* (basionym of *Pseudomonas aeruginosa*), proved the best descriptor.

Since the mid-1980s, certain members of the *Pseudomonas* genus have been applied to cereal seeds or applied directly to soils as a way of preventing the growth or establishment of crop pathogens. This practice is generically referred to as biocontrol. The biocontrol properties of *P. fluorescens* and *P. protegens* strains (CHA0 or Pf-5 for example) are currently best-understood, although it is not clear exactly how the plant growth-promoting properties of *P.*

*fluorescens* are achieved (Shavyrina *et al.*, 2001). Theories include: the bacteria might induce systemic resistance in the host plant, so it can better resist attack by a true pathogen; the bacteria might outcompete other (pathogenic) soil microbes, e.g. by siderophores giving a competitive advantage at scavenging for iron; the bacteria might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide. Experimental evidence supports all of these theories (Singh and Singh, 2001). Other notable *Pseudomonas* species with biocontrol properties include *P. chlororaphis*, which produces a phenazine-type antibiotic active agent against certain fungal plant pathogens, and the closely related species *P. aurantiaca*, which produces di-2,4-diacetylfluoroglucylmethane, a compound antibiotically active against Gram-positive organisms.

#### **2.4.2 *Pseudomonas putida***

*Pseudomonas putida* is a Gram-negative, rod-shaped, saprotrophic soil bacterium. Based on 16S rRNA analysis, *P. putida* was taxonomically confirmed to be a *Pseudomonas* species (*sensu stricto*) and placed, along with several other species, in the *P. putida* group, to which it lends its name. A variety of *P. putida*, called multi-plasmid hydrocarbon-degrading *Pseudomonas*, is the first patented organism in the world (Vessey, 2013). It demonstrates a very diverse metabolism, including the ability to degrade organic solvents such as toluene. This ability has been put to use in bioremediation, or the use of microorganisms to degrade environmental pollutants. Use of *P. putida* is preferable to some other *Pseudomonas* species capable of such degradation, as it is a safe species of bacteria, unlike *P. aeruginosa*, for example, which is an opportunistic human pathogen (Xiong, 2008).

The diverse metabolism of wild-type strains of *P. putida* may be exploited for bioremediation; for example, it has been shown in the laboratory to function as a soil inoculant to remedy naphthalene-contaminated soils. *P. putida* is capable of converting styrene oil into the

biodegradable plastic PHA (Wu *et al.*, 2015). This may be of use in the effective recycling of polystyrene foam, otherwise thought to be not biodegradable. *P. putida* has demonstrated potential biocontrol properties, as an effective antagonist of damping off diseases such as Pythium and Fusarium. *P. putida* amenability to genetic manipulation has allowed it to be used in the synthesis of numerous organic pharmaceutical and agricultural compounds from various substrates (Yanni *et al.*, 2014).

### **2.4.3 *Pseudomonas fluorescens***

*Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium. *P. fluorescens* has multiple flagella. It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration (Faisal and Hasnain, 2016). Optimal temperatures for growth of *P. fluorescens* are 25-30°C. It tests positive for the oxidase test. It is also a nonsaccharolytic bacterial species. Heat-stable lipases and proteases are produced by *P. fluorescens* and other similar pseudomonads. These enzymes cause milk to spoil, by causing bitterness, casein breakdown, and ropiness due to production of slime and coagulation of proteins. Some *P. fluorescens* strains (CHA0 or Pf-5, for example) present biocontrol properties, protecting the roots of some plant species against parasitic fungi such as *Fusarium* or the oomycete *Pythium*, as well as some phytophagous nematodes (De-Pee, 2014)

It is not clear exactly how the plant growth-promoting properties of *P. fluorescens* are achieved; theories include (Casanovas *et al.*, 2003):

- The bacteria might induce systemic resistance in the host plant, so it can better resist attack by a true pathogen.

- The bacteria might outcompete other (pathogenic) soil microbes, e.g., by siderophores, giving a competitive advantage at scavenging for iron.
- The bacteria might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

To be specific, certain *P. fluorescens* isolates produce the secondary metabolite 2,4-diacetylphloroglucinol (2,4-DAPG), the compound found to be responsible for antiphytopathogenic and biocontrol properties in these strains (Caba *et al.*, 2010). The *phl* gene cluster encodes factors for 2,4-DAPG biosynthesis, regulation, export, and degradation. Eight genes, *phIHGFACBDE*, are annotated in this cluster and conserved organizationally in 2,4-DAPG-producing strains of *P. fluorescens*. Of these genes, *phlD* encodes a type III polyketide synthase, representing the key biosynthetic factor for 2,4-DAPG production. *PhlD* shows similarity to plant chalcone synthases and has been theorized to originate from horizontal gene transfer (Chen *et al.*, 2009). Phylogenetic and genomic analysis, though, has revealed that the entire *phl* gene cluster is ancestral to *P. fluorescens*, many strains have lost the capacity, and it exists on different genomic regions among strains.

Some experimental evidence supports all of these theories, in certain conditions; a good review of the topic is written by Haas and Defago. Several strains of *P. fluorescens*, such as Pf-5 and JL3985, have developed a natural resistance to ampicillin and streptomycin. These antibiotics are regularly used in biological research as a selective pressure tool to promote plasmid expression (Hu *et al.*, 2016). The strain referred to as Pf-CL145A has proved itself a promising solution for the control of invasive zebra mussels and quagga mussels (*Dreissena*). This bacterial strain is an environmental isolate capable of killing >90% of these mussels by intoxication (i.e., not infection), as a result of natural product(s) associated with their cell

walls, and with dead Pf-145A cells killing the mussels equally as well as live cells. Following ingestion of the bacterial cells mussel death occurs following lysis and necrosis of the digestive gland and sloughing of stomach epithelium (Edwards *et al.*, 2013). Research to date indicates very high specificity to zebra and quagga mussels, with low risk of nontarget impact. Pf-CL145A has now been commercialized under the product name Zequanox, with dead bacterial cells as its active ingredient. Recent results showed the production of the phytohormone cytokinin by *P. fluorescens* strain G20-18 to be critical for its biocontrol activity by activating plant resistance (Edwards *et al.*, 2013).

By culturing *P. fluorescens*, mupirocin (an antibiotic) can be produced, which has been found to be useful in treating skin, ear, and eye disorders. Mupirocin free acid and its salts and esters are agents currently used in creams, ointments, and sprays as a treatment of methicillin-resistant *Staphylococcus aureus* infection. *P. fluorescens* demonstrates hemolytic activity, and as a result, has been known to infect blood transfusions (Choudhury and Kennedy, 2014). *P. fluorescens* is an unusual cause of disease in humans, and usually affects patients with compromised immune systems. From 2004 to 2006, an outbreak of *P. fluorescens* in the United States involved 80 patients in six states. The source of the infection was contaminated heparinized saline flushes being used with cancer patients. *P. fluorescens* produces phenazine, phenazine carboxylic acid, 2,4-diacetylphloroglucinol and the MRSA-active antibiotic mupirocin (Faisal and Hasnain, 2015)

## **2.5 Plant Growth-Promoting Rhizobacteria (PGPR)**

Plant growth-promoting rhizobacteria (PGPR) were first defined by Sharma *et al.* (2013) to describe soil bacteria that colonize the roots of vegetation following inoculation onto seed and that beautify plant boom. The following are implicit inside the colonization process: ability to live to tell the tale inoculation onto seed, to multiply within the spermosphere (place

surrounding the seed) in reaction to seed exudates, to connect to the foundation surface, and to colonize the growing root machine. The ineffectiveness of PGPR inside the discipline has frequently been attributed to their incapability to colonize plant roots (Zaidi *et al.*, 2009). A diffusion of bacterial traits and specific genes contribute to this procedure, but just a few were recognized. These include motility, chemotaxis to seed and root exudates, manufacturing of pili or fimbriae, manufacturing of precise cell surface additives, ability to use unique components of root exudates, protein secretion, and quorum sensing. The era of mutants altered in expression of these developments is aiding our know-how of the correct role each one performs inside the colonization technique (Parmar and Sindhu, 2013).

Plant growth-promoting rhizobacteria (PGPR) had been well known to gain vegetation thru several direct and indirect mechanisms specifically biological nitrogen fixation, phosphate and potassium solubilization, manufacturing of plant increase regulators, siderophore, hydrolyzing enzymes and many extra. Those beneficial microorganisms additionally act as biocontrol marketers towards pests and diseases through inducing plant systemic resistance (Kumar and Dubey, 2012). Regardless of the huge superb effects, there are inconsistent troubles in terms of utility of PGPR on rice flowers. The most essential useful impact is derived from organic nitrogen fixation (BNF) that may reduce chemical or inorganic N-fertilizer usage, a big contribution seeing that rice cultivation requires excessive amount of N-fertilizer. Its miles envisioned that rice plants put off 19.4 kg N for every tonne of rice grain yield (Han and Lee, 2006). Moreover, urea as the commonly used N-fertilizer has low plant uptake efficiency, frequently at most effective 30-40% in spite of the strength-in depth manufacturing tactics which involve herbal gas, a non-renewable resource.

The most substantial research of N<sub>2</sub>-solving plant-microorganism interaction have been on legume-rhizobia symbiosis, in which the bacteria restoration atmospheric nitrogen as endosymbionts interior root nodules in a nutrient-rich and oxygen-managed micro-

environment. This symbiosis is a number-unique interplay whereby the rhizobia only nodulate one hosts specie and very few pass-inoculations. Currently, researchers began to mission into the opportunity of inoculating rhizobia on non-legumes including rice and there have been scattered reports of achievement, particularly via enhancement of rice seedling growth and grain yield (Liu *et al.*, 2012). However, the mechanisms remain unclear, and it becomes no longer via formation of root nodules as inside the rhizobia-legume symbiosis. The workers have postulated that it may be due to the rhizobia acting and appearing like PGPR in supplying the beneficial outcomes inclusive of phytohormone production, phosphate solubilization and development in soil N assimilation. Ma (2015) remoted photosynthetic *Bradyrhizobium* sp. pressure as natural endophyte in African wild rice (*Oryza breviligulata*) and through acetylene reduction assay (ARA) and greenhouse studies confirmed that this stress produced a sizable level of N<sub>2</sub> fixing pastime with 20% will increase in shoot and grain yields. In comparison, numerous other researchers who used ARA and N dilution techniques of their research concluded that the useful results of rhizobial inoculation on rice was now not normally thru BNF but via physiological modifications in rice growth and root morphology (Arora *et al.*, 2013).

Currently, there was extremely good hobby within the application of PGPR and rhizobial strains as multi-pressure inocula for vegetation to benefit from their unique beneficial traits. BNF with the aid of a few diazotrophic bacteria like *Azotobacter*, *Clostridium*, *Azospirillum*, *Herbaspirillum* and *Bukholderia* can alternative a considerable amount of N-fertilizer, while Rhizobium can promote physiological growth or improve root morphology of rice flora. It's miles hypothesized that this multi-strain biofertilizer inoculum can assist sell plant increase and rice grain yield probable through BNF alongside numerous other recognised beneficial effects of PGPR and rhizobia. This may benefit the rice vegetation through progressed increase and yield whilst minimizing the use of chemical N-fertilizer. Hence, a discount in

production costs and environmental problems and a merchandising of a green and sustainable agriculture will appear (Schwyn and Neilands, 2015).

## **2.6 Plant Growth Promotion: Mechanism of Action**

### **2.6.1 Nitrogen fixation**

Nitrogen is a critical element for all styles of existence and it is the maximum vital nutrient for plant increase and productivity. Despite the fact that the nitrogen offers 78 % of the atmosphere, it remains unavailable to the flowers. Lamentably no plant species is capable for solving atmospheric dinitrogen into ammonia and dissipate it directly for its increase (Cornelis, 2010). For that reason, the atmospheric nitrogen is transformed into plant utilizable paperwork by way of biological nitrogen fixation (BNF) which adjustments nitrogen to ammonia by means of nitrogen solving microorganisms the usage of a complex enzyme system referred to as nitrogenase. Plant growth selling rhizobacteria have the potential to fix atmospheric nitrogen and offer it to flora by using two mechanisms: symbiotic and non-symbiotic. Symbiotic nitrogen fixation is a mutualistic relationship among a microbe and the plant. The microbe first enters the root and in a while form nodule in which nitrogen fixation occurs. Rhizobia are an enormous organization of rhizobacteria which have the capability to put symbiotic interactions via the colonization and formation of root nodules with leguminous vegetation, where nitrogen is fixed to ammonia and make it available for the plant (Sujatha and Ammani, 2013). The plant growth promoting rhizobacteria extensively presented as symbionts are *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* with leguminous plant life, *Frankia* with non-leguminous trees and shrubs.

However, non-symbiotic nitrogen fixation is accomplished by using free dwelling diazotrophs and this could stimulate non-legume vegetation boom such as radish and rice.

Non-symbiotic Nitrogen solving rhizospheric bacteria belonging to genera which includes *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria (Sharma *et al.*, 2003). The genes for nitrogen fixation, referred to as *nif* genes are located in both symbiotic and unfastened dwelling structures. Nitrogenase (*nif*) genes encompass structural genes, concerned in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and feature of the enzyme. Inoculation with the aid of organic nitrogen solving plant increase promoting rhizobacteria on crop provide an incorporated method for sickness control, increase promoting interest, preserve the nitrogen degree in agricultural soil (Miransari and Smith, 2014).

### **2.6.2 Phosphate Solubilization**

Phosphorus is the maximum vital key element inside the vitamins of plant life, subsequent to nitrogen (N). It performs a vital role in certainly all main metabolic strategies in plant such as photosynthesis, strength switch, signal transduction, macromolecular biosynthesis and respiration. it's far abundantly to be had in soils in each organic and inorganic paperwork (Spaepen and Vanderleyden, 2011). Plants are unable to applied phosphate because 95-99% phosphate present within the insoluble, immobilized, and brought on form. Flowers take in phosphate best in soluble forms, the monobasic ( $H_2PO_4$ ) and the diabasic ( $HPO_4^{2-}$ ) ions. Plant increase selling rhizobacteria present in the soil employ extraordinary techniques to utilize unavailable kinds of phosphorus and in turn also assist in making phosphorus to be had for flora to take in. the principle phosphate solubilization mechanisms employed by plant boom selling rhizobacteria include (Shilev, 2013): (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions,  $CO_2$ , (2) liberation of extracellular enzymes (biochemical phosphate mineralization) and (3) the discharge of

phosphate at some point of substrate degradation (organic phosphate mineralization). Phosphate solubilizing PGPR blanketed in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* have attracted the eye of agriculturists as soil inoculums to enhance plant boom and yield. however, the useful outcomes of the inoculation with phosphate solubilizing bacteria used on my own or in mixture with other rhizospheric microbes have been also stated (Youssef and Eissa, 2014).

### 6.63.3 Potassium solubilization

Potassium is the third fundamental crucial macronutrient for plant boom. The concentrations of soluble potassium within the soil are commonly very low and greater than ninety% of potassium inside the soil exists in the form of insoluble rocks and silicate minerals. moreover, because of imbalanced fertilizer application, potassium deficiency is turning into one of the important constraints in crop manufacturing. without ok potassium, the plant life can have poorly developed roots, grow slowly, produce small seeds and have lower yields (Joshi et al., 2006). This emphasised the search to discover an opportunity indigenous supply of potassium for plant uptake and to keep potassium reput in soils for maintaining crop production. Plant boom selling rhizobacteria are capable of solubilize potassium rock via manufacturing and secretion of organic acids. Potassium solubilizing plant boom promoting rhizobacteria which includes *Acidothiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Burkholderia*, *Paenibacillus* sp. and *Pseudomonas* has been pronounced to release potassium in on hand form from potassium bearing minerals in soils. for this reason, software of potassium solubilizing plant increase promoting rhizobacteria as biofertilizer for agriculture development can reduce the usage of agrochemicals and assist ecofriendly crop production (Hiltner, 2014).

### **2.6.3 Siderophore production**

Iron is an important micronutrient for nearly all organisms within the biosphere. regardless of the reality that iron is the fourth maximum considerable detail in the world, in aerobic soils, iron is not effectively assimilated by means of both bacteria or flora due to the fact ferric ion or  $Fe^{+3}$ , that is the foremost form in nature, is handiest sparingly soluble so that the amount of iron to be had for assimilation by dwelling organisms is extraordinarily low (Akhtar *et al.*, 2012). Microorganisms have evolved specialized mechanisms for the assimilation of iron, including the manufacturing of low molecular weight iron-chelating compounds known as siderophores, which delivery this element into their cells. Siderophores are divided into three fundamental households relying on the feature practical group, i.e. hydroxamates, catecholates and carboxylates. At present extra than 500 one-of-a-kind sorts of siderophores are regarded, of which 270 were structurally characterized (Kloepper *et al.*, 2000). Siderophores had been implicated for each direct and indirect enhancement of plant growth by plant growth selling rhizobacteria. The direct advantages of bacterial siderophores at the boom of plants were proven by way of the usage of radiolabeled ferricsiderophores as a sole supply of iron confirmed that plants are capable of soak up the categorized iron by a massive number of plant boom selling rhizobacteria including *Aeromonas*, *Azadirachta*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Streptomyces* sp. and more suitable chlorophyll stage in comparison to un inoculated plant life (Ahemad and Khan, 2009).

### **2.6.4 Phytohormone Production**

A wide range of microorganisms discovered within the rhizosphere are able to produce substances that modify plant boom and development. Plant increase promoting rhizobacteria produce phytohormones including auxins, cytokinins, gibberellins and Ethylene can affect

cellular proliferation within the root structure with the aid of overproduction of lateral roots and root hairs with a next increase of nutrient and water uptake (Sivasakhti *et al.*, 2014).

### **2.6.5 Indole Acetic Acid (IAA)**

Among plant growth regulators, indole acetic acid (IAA) is the maximum commonplace natural auxin located in flowers and its nice impact on root increase. as much as eighty% of rhizobacteria can synthesize indole acetic acid (IAA) colonized the seed or root surfaces is proposed to behave together with endogenous IAA in plant to stimulate cellular proliferation and beautify the host's uptake of minerals and nutrients from the soil (Sagar *et al.*, 2012). Indole acetic acid affects plant cellular division, extension, and differentiation; stimulates seed and tuber germination; will increase the price of xylem and root improvement; controls methods of vegetative increase; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; influences photosynthesis, pigment formation, biosynthesis of diverse metabolites, and resistance to demanding situations. Tryptophan is an amino acid generally observed in root exudates, has been diagnosed as predominant precursor molecule for biosynthesis of IAA in microorganism (Maheshwari *et al.*, 2012).

The biosynthesis of indole acetic acid by means of plant increase selling rhizobacteria includes formation thru indole-three- pyruvic acid and indole-3-acetic aldehyde, which is the most not unusual mechanism in bacteria like *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Enterobacter* and *Klebsiella*. Root growth advertising by means of the free living PGPR e.g., *Alkaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter dizotrophicous*, species of *Azospirillum*, *Pseudomonas* and *Xanthomonas* sp. has been associated with low level of IAA secretion (Viveros *et al.*, 2010). but, microbially produced phytohormones are extra powerful because of the purpose that the edge between inhibitory and stimulatory stages

of chemically produced hormones is low, at the same time as microbial hormones are more effective through virtue in their non-stop slow release.

### 2.6.6 Cytokinins and Gibberellins

several plant increase selling rhizobacteria *Azotobacter* sp., *Rhizobium* sp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Paenibacillus polymyxa* can produce cytokinins or gibberellins or each can produce both cytokinins or gibberellins or both for plant growth promotion (Ahemad and Kibret, 2014). some strains of phytopathogens also can synthesize cytokinins. However, it seems that plant boom promoting rhizobacteria produce decrease cytokinin ranges as compared to phytopathogens so that the effect of the plant growth selling rhizobacteria on plant increase is stimulatory while the impact of the cytokinins from pathogens is inhibitory. Ethylene is a key phytohormone has a huge variety of biological sports can have an effect on plant growth and development in a large variety of various approaches inclusive of selling root initiation, inhibiting root elongation, selling fruit ripening, promoting decrease wilting, stimulating seed germination, promoting leaf abscission, activating the synthesis of other plant hormones (Kloepper and Schroth, 2011). The excessive awareness of ethylene induces defoliation and other cellular techniques that could cause decreased crop performance.

The enzyme 1-aminocyclopropane-1 carboxylic acid (ACC) is a pre-considered necessary for ethylene manufacturing, catalyzed via ACC oxidase. Bhardwaj et al. (2014) reported improved nodule wide variety, nodule dry weight, sparkling biomass, grain yield, straw yield, and nitrogen content in grains of lentil as a result of reducing of the ethylene production through inoculation with plant increase selling lines of *Pseudomonas* sp. containing ACC deaminase together with *R. leguminosarum*. Presently, bacterial strains displaying ACC deaminase interest were identified in a extensive variety of genera inclusive of *Acinetobacter*,

Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia and Rhizobium (Arora et al., 2012).

### **2.6.7 Induced Systemic Resistance (ISR)**

Induced resistance may be defined as a physiological nation of improved shielding ability elicited in response to precise environmental stimuli and consequently the plant's innate defenses are potentiated towards subsequent biotic demanding situations. Biopriming vegetation with a few plant growth selling rhizobacteria also can offer systemic resistance towards a extensive spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even harm due to insects and nematodes may be decreased after application of plant growth promoting rhizobacteria (Gaby and Buckley, 2012). Moreover, induced systemic resistance entails jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's protection responses against an expansion of plant pathogens. Many person bacterial components set off precipitated systemic resistance including lipopolysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2, 4-diacetylphloroglucinol, homoserine lactones, and volatiles like, acetoin and 2, 3-butanediol.

### **2.6.8 Exopolysaccharides production**

Positive microorganisms synthesize a wide spectrum of multifunctional polysaccharides which include intracellular polysaccharides, structural polysaccharides, and extracellular polysaccharides (Zahran, 2001). Production of exopolysaccharides is generally critical in biofilm formation; root colonization can have an effect on the interplay of microbes with roots appendages. effective colonization of plant roots by means of EPS-producing microbes enables to hold the free phosphorous from the insoluble one in soils and circulating essential nutrient to the plant for proper increase and improvement and defensive it from the assault of

foreign pathogens (Vessey, 2013). Other innumerable features finished with the aid of EPS producing microbes represent protective from desiccation, safety towards stress, attachment to surfaces plant invasion, and plant protection reaction in plant microbe interactions. Plant boom promoting rhizobacterial producing exopolysaccharides are particularly important in selling plant boom because of paintings as a lively signal molecule at some stage in useful interactions, and provide protection response at some point of infection technique. a few plant increase promoting rhizobacterial generating exopolysaccharides can also bind cations, along with Na<sup>+</sup>suggesting a position in mitigation of salinity strain via reducing the content of Na<sup>+</sup> available for plant uptake (Reed *et al.*, 2011).

## **2.7 Amaranthus species**

*Amaranthus*, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial plants. Some amaranth species are cultivated as leaf vegetables, pseudocereals, and ornamental plants. Most of the *Amaranthus* species are summer annual weeds and are commonly referred to as pigweed. Catkin-like cymes of densely packed flowers grow in summer or autumn. Approximately 60 species are recognized, with inflorescences and foliage ranging from purple, through red and green to gold. Members of this genus share many characteristics and uses with members of the closely related genus *Celosia*.

Uncooked amaranth grain is 12% water, 65% carbohydrates (including 7% dietary fiber), 14% protein, and 7% fat. A 100 grams (3.5 oz) reference amount of uncooked amaranth grain provides 371 calories, and is a rich source (20%) of protein, dietary fiber, pantothenic acid, vitamin B6, folate, and several dietary minerals. Uncooked amaranth is particularly rich in manganese, phosphorus, magnesium, iron, and selenium. Cooking decreases its nutritional value substantially across all nutrients, with only dietary minerals remaining at moderate levels. Cooked amaranth leaves are a rich source of vitamin A, vitamin C, calcium, and

manganese, with moderate levels of folate, iron, magnesium, and potassium. Amaranth grain contains phytochemicals that are not defined as nutrients and may be antinutrient factors, such as polyphenols, saponins, tannins, and oxalates. These compounds are reduced in content and antinutrient effect by cooking.

### **2.7.1 Amaranthus hybridus**

*Amaranthus hybridus* commonly called green amaranth, slim amaranth, smooth amaranth, smooth pigweed, or red amaranth, is a species of annual flowering plant. It is a weedy species found now over much of North America and introduced into Europe and Eurasia. *A. hybridus* originates probably from lowland tropical South and Central America and was introduced into other warmer parts of the world from about 1700 AD onwards (Zakry *et al.*, 2012). At present it occurs in all tropical and subtropical regions, including tropical Africa, often gregariously and as a weed. It is sometimes found in temperate zones as well. It is rarely cultivated.

### **2.7.2 Botanical Description**

*Amaranthus hybridus* grows from a short taproot and can be up to 2.5 m in height. It is a glabrous or glabrescent plant. *A. hybridus* is well distributed in eastern North America. It has been reported to have been found in every state except Wyoming, Utah, and Alaska. It is also found in many provinces of Canada, and in parts of Mexico, the West Indies, Central America, and South America. It has been naturalized in many places of warmer climate (Kovacs *et al.*, 2009). It grows in many different places, including disturbed habitats. Although easily controlled and not particularly competitive, it is recognized as a harmful weed of North American crops. The plant was used for food and medicine by several Native American groups and in traditional African medicine.

### 2.7.3 Scientific Classification

Kingdom: Plantae

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Amaranthus*

Species: *A. hybridus*

Binomial name: *Amaranthus hybridus* L.

### 2.7.4 Uses

In tropical Africa and elsewhere *A. hybridus* leaves and young plants are collected for home consumption as a cooked, steamed or fried vegetable, especially during periods of drought. Leaves are occasionally found for sale on markets. *A. hybridus* is also used as forage and said to increase the yield of milk in cattle (Leonard *et al.*, 2004). The root is known as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea and is also applied as an emmenagogue and antipyretic. In many countries, including those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of eczema, burns, wounds, boils, earache and haemorrhoids.

### 2.8 *Amaranthus viridis*

*Amaranthus viridis* is a cosmopolitan species in the botanical family Amaranthaceae and is commonly known as slender amaranth or green amaranth (Choudhury and Kennedy, 2014). *Amaranthus viridis* is an annual herb with an upright, light green stem that grows to about 60–80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3–6

cm long, 2–4 cm wide, with long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers with 3 stamens.

### 2.8.1 Scientific Classification

Kingdom: Plantae

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Amaranthus*

Species: *A. viridis*

Binomial name: *Amaranthus viridis* L.

### 2.8.2 Uses of *Amaranthus viridis*

*Amaranthus viridis* is eaten as a boiled green or as a vegetable in many parts of the world. In the Northeastern Indian state of Manipur, it is known as cheng-kruk; it is also eaten as a vegetable in South India, especially in Kerala, where it is known as kuppacheera. It is a common vegetable in Bengali cuisine, where it is called note shak (leafy vegetable) (Zakry *et al.*, 2012). It is a very common vegetable used in Odia Cuisine as Saaga, namely as Kosila Saaga or Marshi Saag in rural areas. It is also eaten as a vegetable in parts of Africa including Nigeria. The leaves of this plant, known as massaagu in Dhivehi, have been used in the diet of the Maldives for centuries in dishes such as mas huni. In the 19th Century *A. viridis*, or green amaranth was an item of food in Australia. The botanist Joseph Maiden wrote in 1889: It is an excellent substitute for spinach, being far superior to much of the leaves of the white beet sold for spinach in Sydney (Casanovas *et al.*, 2003). Next to spinach it seems to be most

like boiled nettle leaves, which when young are used in England, and are excellent. This amarantus should be cooked like spinach, and as it becomes more widely known, it is sure to be popular, except amongst persons who may consider it beneath their dignity to have anything to do with so common a weed.

Green amaranth also has clusters of nutty edible seeds, which can be eaten as snacks or used in biscuits (Manful *et al.*, 2007). A portage can be made by boiling the leaves in water with yam. Unlike other amaranths, the seeds can be easily harvested by scraping the ripe spikes of seeds between the fingers. Green Amaranth can contain up to 38% protein by dry weight. The leaves and seeds contain lysine, an essential amino acid as such it is used as a medicinal herb in traditional Ayurvedic medicine; under the Sanskrit name Tanduliya (Miche and Balandreau, 2013).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Experimental Area**

The field study of the experiment was conducted in the botanical garden of the department of Plant biology and biotechnology, University of Benin, Edo state. The garden characterized by dominant species including *Agerantum conyzoides*, *Eleusine indica* among others.

## **3.2 Land Preparation**

The study began with the cutout of an experimental plot of 2.5m by 3.5m. The experimental plot was cleared out before experimental bowls were purchased and put on the plot.

## **3.3 Research Methodology**

### **3.3.1 Collection of Microbial Sample**

*Pseudomonas fluorescens* and *P. putida* were collected from the collection center from stock culture at the department of microbiology, university of Benin.

### **3.3.2 Preparation of Microbial Sample**

The bacteria inoculums were prepared by direct colony suspension where a small volume of sterile normal saline was poured into a McCartney bottle to which general colonies of the test organisms taken directly from the plate, was emulsified and the suspension adjusted to match the 0.2 McFarland standard which has similar appearance of an overnight both culture by adding normal saline. Preparation of McFarland Turbidity Standard McFarland standards are used as turbidity standards in the preparation of bacterial suspensions so that the number of bacteria will be within a given range. 0.2 McFarland standard is prepared by mixing 0.2 ml of 1% barium chloride dehydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ), with 9.8 ml of 1% sulphuric acid solution ( $\text{H}_2\text{SO}_4$ ) to form a barium sulphate precipitate, which causes turbidity in the solution. A small volume of the turbid solution was transferred to McCartney bottle of the same type that was used to prepare the test and of control inocula. This was stored in a sterile- dark room temperature. Exactly 0.2 McFarland gives an equivalent approximate density of bacteria  $6.0 \times 10^8$  cfu<sup>16</sup>.

### **3.4 Collection of Seed Sample**

The seed of *Amaranthus hybridus* and *Amaranthus viridis* used for this study were obtained from the National Horticultural Research Institute (NIHORT), Ibadan.

#### **3.4.1 Preparation of Seed Samples**

Seeds were subjected to viability by floatation method, which is done by soaking the seedings in a container. The seeds that floats on water are taken as inviable, while those that sinks are taken as viable (Ehilen *et al.*, 2017). The test seeds were deliberately not sterilized for the purpose of maintaining the microbial integrity of the seed. This is to avoid complication of technique when sold to the farmer, should the study give a positive result.

#### **3.4.2 Collection of Soil Sample**

The humus soil used in the experiment were dug out from a fallow land especially at the base of banana plant and was is characteristically dark in colour while the ferruginous soil used for the experiment was collect at Capitol, University of Benin and is characteristically red. Care was taken to ensure that the soil were collected between 0-10cm height of the soil to ensure the collection of top soil. The soils were confirmed humus and ferruginous by the department of soil science, university of Benin.

#### **3.4.3 Preparation of Soil Sample**

About 40 grams of the soil sample was collected to check for physicochemical parameters. The soils were deliberately not sterilized for the purpose of maintaining the microbial integrity of the soil. This is to avoid complication of technique when sold to the farmer, should the study give a positive result.

### **3.5 Field Experiment**

Four perforated bowls were filled with 5kg of red soil each, another set of 4 perforated bowls were filled with 5kg of loamy soil. Each of the bowls were well watered and allowed to stay till the next day. On the morrow, 5grams of the viable *Amaranthus* seeds were planted in each bowl by sprinkling, after which the surface of the soil was streaked with a small piece of stick to ensure the seeds were evenly distributed in the bowl. The whole set up was done in three replicates. Watering was done every day with 300ml of water on a daily basis except for days it rained. After two weeks after germination the seedlings were rhizo-injected with 25ml

#### **3.5.1 Treatments of the Field Experiment**

The field experiment is made up of eight treatments, four was on the red soil while the remaining four were on the loamy soil.

The treatments took effect after two weeks of germination. One of the red soil was rhizo-injected with 50ml of the *P. fluorescens* suspension solution and was labelled RSF, the second was rhizo-injected with 50ml of *P. putida* and was labelled RSP, the third was rhizo-injected with the suspension containing the mixture of both the fluorescens and the putida species of Pseudomonas and was so labelled PSFP while the last was left unrhizo-injected and so labelled RS.

The same was done for the black soil and has the label BSF, BSP, BSFP and BS.

#### **3.5.2 Germination Study**

Germination study was carried as an in-vitro because the plant sown in the ferruginous soil could not survived beyond 45 days irrespective of treatment on the field, For the germination study, soil solution was made by mixing water and soil in a ratio of one to one, after which it was subjected to filtration using wattman filter paper to obtain the soil solution.

### **3.5.3 Germination Study Treatments**

The germination study was divided into 3 sections namely; the first with the filtrate of red soil, the second with the filtrate of black soil and the third with ordinary water and they are labelled RS, BS and W respectively.

### **3.5.4 RS Treatment**

Four petri dishes lined with filter paper wet with 2ml of the filtrate from the red soil solution were used for the RS treatment. 50 Seeds soaked for 24 hours and 3 hours were planted on each side of the petri dishes and was demarcated by a clear space visible enough to serve as demarcation. The seeds soaked for 24 hours were on the right and was labelled Y standing for 'yesterday' while those soaked for 3 hours were on the left and labelled T standing for 'today'. This arrangement was done for each of the soaking treatments, with the seeds soaked in *P. fluorescens* planted in the first petri dish and labelled RSF, the seeds soaked in *P. putida* suspension was planted in the second petri dish and was labelled RSP, the seeds soaked in the mixture of *P. fluorescens* and *P. putida* was planted in the third petri dish and was labelled RSFP while the seeds soaked in water was planted in the fourth petri dish and was labelled RSW and served as the control for the other three.

### **3.5.5 BS Treatment**

The same method was use as described in section 2.5.1.1 with the equivalent petri dishes labelled BSF, BSP, BSFP and BSW.

### **3.5.6 W Treatment**

The same method was use as described in section 2.5.1.1 with the equivalent petri dishes labelled WF, WP, WFP and WW.

### **3.6 Measurement of Experimental Parameter**

#### **3.6.1 Height**

The height of the plants was measured using meter rule and tape rule when necessary, every 5 days up to the termination of the experiment at the 65<sup>th</sup> day. The height of the plant was recorded in centimeter.

#### **3.6.2 Stem Girth**

Stem girth was determined by tying a tiny rope round the stem of the plant. The length of the tread is taken as the circumference of the stem. The girth is determined by calculating the diameter from the circumference.

Circumference =

d=

d= diameter

#### **3.6.3 Leaf area**

Before calculating the leaf area, the length of the leaf and the breadth were measured using meter rule. The leaf area was calculated by multiplying the length and the breadth by the leaf constant.

Area = leaf length × leaf breadth × 0.75 (cm<sup>2</sup>)

#### **3.6.4 Number of leaves**

The number of leaves was determined by counting the total number of the leaves on the plant.

### **3.6.5 Length of leave stalk**

The length of leave stalk was measured using meter rule directly on three prominent leaves followed by taking average

### **3.6.6 Length of internode**

The length of internode was determined by taking the average of 3 internodes measured using meter rule.

### **3.6.7 Root length**

The length of the root was determined by uprooting and measuring the length of the primary and secondary root length.

### **3.6.8 Dry weight of root**

This is achieved by uprooting the plant, followed by washing, and drying in the oven. After drying the root are weighed on the sensitive weighing balance.

### **3.6.9 Day to flowering**

This is recorded as the day the plants in each treatment starts flowering.

### **3.6.10 Maximum length of inflorescence**

This is recorded as the maximum length the flower attained before termination of the experiment

### **3.2.6.11 Rate of plant parameter development**

The rate of development of parameter is determined using the formula

Rate =

P=measured parameter per unit measurement

t=time during which parameter was measured

n= number of periods

### **2.6.12 Time per leaf formation**

The time taken for the formation of one leaf is calculated using the formula

$t_x =$

$t_x$ = time taken for the production of one leaf

l= number of leaves per unit time

n= number of periods

## **3.7 Soil Physicochemical Analyses**

Soils were dried at ambient temperature (22 - 25°C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of selected soil physicochemical properties and the heavy metal fractions as well as PAH.

### **3.7.1 Total organic carbon (TOC) and total organic matter (TOM) contents**

Half a gram (0.5 g) of each air-dried soil sample was put into a conical flask and 2.5 ml of 1N potassium dichromate solution  $K_2Cr_2O_7$  was added and swirled gently to disperse the

sample in the solution. Five millilitres (5 ml) of concentrated tetraoxosulphate (VI) acid was added rapidly, into the flask and swirled gently until sample and reagents were mixed and finally swirled vigorously for about a minute. The flask was allowed to stand in a fume cupboard for 30 minutes. Five to ten (5 to 10) drops of the indicator were added and the solution titrated with 0.5N FeSO<sub>4</sub> to maroon colour. A blank determination was carried out to standardize the dichromate (Nelson and Sommers, 1982). TOC and TOM contents were calculated as follows (Osuji and Nwoye, 2007):

TOC ( %) =

Where

w= Weight of sample (g)

meq K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> = 1N X 2.5 ml

meq FeSO<sub>4</sub> = 0.5 N X Volume of titrant in ml

0.03 = Milliequivalent weight of carbon

1.30 = Correction factor

TOM ( %) = TOC ( %) x 1.724

Where: 1.724 = Conversion Factor; [i.e. %TOM = %TOC x 100 /58

since TOC is 58 % of TOM

### **3.7.2 Determination of Soil Nitrogen**

Digestion of Soil Nitrogen by Micro-Kjeldahl Digestion

Nitrogen in the soil was determined by Kjeldahl digestion, and the resulting ammonium ion measured colorimetrically. Elements such as iron and manganese, which may interfere in the alkaline medium during colorimetric determination, were first complexed with sodium potassium tartrate. The Ammonia was determined colorimetrically as the indophenol blue complex by reaction with alkaline sodium phenate and sodium hypochlorite.

Some 0.2 g of finery ground soil was weighed into 30 ml Kjeldahl digestion flask; and one tablet of catalyst and 4.0 ml of conc. H<sub>2</sub>S<sub>4</sub> were added to it. This was properly shaken to ensure complete mixing of the soil and catalyst mixture. The flask was placed on the heater and digested for about 45 minutes. At the completion of the digestion, the mixture was clear. The flask was then removed from the heater. It was cooled until just warm to touch, and then 10 ml of distilled water was added. It was important that the mixture in the Kjeldahl flask did not solidify before the addition of water, as it was time-consuming re-dissolving the solids. Solution was decanted through a Whatman filter paper No. 42 into 100 volumetric flasks. The Kjeldahl flask was washed with 3 small aliquots of distilled water, adding all the washings into the volumetric flask via the filter paper and made up to volume. Nitrogen was determined in the filtrate.

### **3.7.3 Determination of Available Phosphorus**

#### **3.7.3.1 Extraction of Available Phosphorus**

An extracting solution (0.03 M NH<sub>4</sub> F in 0.025 M HCL) was first prepared by dissolving 1.1 g of NH<sub>4</sub>F in water and adding 4.16 ml of 6 M HCL and then made up to 1 litre. 5 g of the soil was weighed into the plastic bottle. 40 ml of the extracting solution (0.03 M NH<sub>4</sub> F in 0.025 M HCL) was added, and was stoppered. This was shaken manually for 1 minute and filtered with Whatman filter paper No. 42. The filtrate was reserved for P determination.

### 3.7.3.2 Determination of Phosphorus

Twelve (12) g of Ammonium Molybdate was dissolved in 250 ml of water. Some 0.2908 g Antimony potassium Tartrate was also dissolved in 100 ml of water. Some 2.5 M H<sub>2</sub>SO<sub>4</sub> was prepared by making 136 ml of cone. H<sub>2</sub>SO<sub>4</sub> to 1 liter. The ammonium molybdate and antimony potassium tartrate were then added to 1000 ml of 2.5 M H<sub>2</sub>SO<sub>4</sub>; mixed thoroughly, made to 2000 ml and stored in plastic container in a cool dark compartment.

A small amount (0.53 g) of Ascorbic Acid was dissolved in 200 ml of reagent as prepared above, and then the mixture was prepared as required since it does not keep for more than 24 hours.

Other reagents required were 0.25 % p-Nitrophenol, 2 M HCL, 2 M NH<sub>4</sub>OH and a P-Standard Stock (100 mg/l) that was prepared by dissolving 0.4394 g of KH<sub>2</sub>PO<sub>4</sub> in water and made to 1 litre. Pipetting 0, 1, 2, 3, 4 and 5 ml from the 100 ml stock solution, intermediate standards of 0, 2, 4, 6, 8, and 10 mg/l were then prepared each in 50 ml flask.

Five (5) ml of the filtrate or supernatant was pipetted into a 50 ml flask, while the pH of the solution was adjusted to 5 by adding 3 drops of the p-nitrophenol, and when a yellow colour was not obtained, some drops of 2 M NH<sub>4</sub>OH were added until yellow. Then 2 M HCL was added drop-wise until colourless (the pH was now between 3 and 5).

Water was added to 30 ml, and then 10 ml of the Ascorbic Acid reagent was added. This was made to volume and read spectrophotometrically at 660 nm.

Calculation:

P(mg/l) =

### **3.7.4 Determination of Iron content**

The determination of iron content of the soil was done using Disodium Dihydrogen Ethylenediamine Tetraacetate Titration as described by Cheng *et al.*, 1952. This method involves digestion using concentrated perchloric acid, followed by titration with versanate solution. The iron content is calculated using the formula

$$= X 100$$

### **3.8 Phytochemical Screening**

The Phytochemical screening were performed on the *Abrus precatorious* leaves extract using standard procedures by Sofowora (1993), Trease and Evans (1989), as well as Odebiyi and Sofowora (1978).

#### **3.8.1 Test for Glycosides**

1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring is required for the presence of glycoside.

#### **3.8.2 Test for Saponins**

0.5g of plant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as a standard.

#### **3.8.3 Test for Flavonoids**

2ml of the extract was boiled with distilled water and filtered. The filtrate was divided into different portions A and B of 5ml each.

- (i) **To portion A:** 10% Lead acetate solution was added in few drops. A yellowish precipitate is indicative of a positive result.
- (ii) **To portion B:** 5ml of 20% NaOH and few drops of dilute HCL were added to the solution. Formation of a colourless solution is indicative of a positive test.

#### **3.8.4 Test for Phenolic compounds**

1ml of the plant extract was added to 5ml of 90% ethanol. In addition, 1 drop of 10% FeCl<sub>3</sub> was added. A pale yellow colouration is indicative of positive test.

#### **3.8.5 Test for Tannins**

To 2ml of the extract, 10ml of distilled water was added and boiled for 5 minutes and then filtered into halves.

- i. To about 2 drops of the filtrate, ferric chloride (FeCl<sub>3</sub>) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.
- ii. To about 5 drops of the filtrate, 2ml dilute HCl was added and boiled for 5 minutes. Red precipitate is required for condensed tannin.

#### **3.8.6 Test for Steroids**

2ml of acetic anhydride was added to 0.5g plant extract in 2ml of dilute H<sub>2</sub>SO<sub>4</sub>. A colour change from violet to blue or green is required for the presence of steroids.

### **3.8.7 Test for Terpenoids (Salkowski test)**

5ml of each extract was mixed in 2ml of chloroform and 3mls of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

### **3.8.8 Test for Alkaloids**

Dragendoff's Wagner's reagent and Picric acid were used to test for alkaloids.

About 1ml of the plant extract was transferred into three different test tubes labelled A, B and C.

- i. To portion A: 2mls of Dragendoff's reagent (made of a mixture of Potassium Bismuth Iodide) was added. Reddish brown precipitate is required for positive test.
- ii. To portion B: 2mls of Wagner's reagent was added. Reddish brown precipitate is indicative for positive test.

To portion C: 2mls of Picric acid was added. A yellowish precipitate test is a positive test.

### **3.9 Determination of total phenolic contents**

The amount of total phenolics in extracts was determined with the Folin- Ciocalteu reagent. Galic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml of galic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.1ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. The Folin-Ciocalteu reagent is sensitive

to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined.

### **3.10 Statistical analysis**

Mean and statistical error of data was calculated (Zar, 1974). Analysis of variance in complete by randomized design was done using the SPSS-16 statistical software, and means were separated by using the Least Significant Difference (Ogbeibu, 2005).

## CHAPTER FOUR

### 4.0

### RESULTS

#### 4.1 Field Studies

Plant parameters at 65 days after sowing just before harvest have been presented on Table 4.1. It must be noted however, that the plant sown in the ferruginous soil could not survive beyond 45 days irrespective of treatment. They also did not attain above a height of 3cm and as such were not reasonable for harvest. Only the plants sown in humus soil, whether rhizo-inoculated or not presented plant parameters that were suitable for harvest time. Plant height ranged from 30.1 – 39.2 cm in *A. hybridus* ( $p=0.293$ ) and 35.3 – 41.6 cm ( $p=0.072$ ) in *A. viridis*, notwithstanding rhizo-inoculation method employed. For *A. hybridus*, Leaf area was significantly higher in plant that were rhizo-inoculated with the test microorganisms compared to the control. Leaf area in the control plant (plant that were not rhizo-inoculated but yet sown in humus soil) was 10.2 cm<sup>2</sup>. However, when the *A. hybridus* plants were rhizo-inoculated with any of the *Pseudomonas putida* or *P. fluorescens* or a combination of both bacteria, leaf area ranged from 15.2 – 21.3 cm<sup>2</sup>. No significant changes in leaf area was reported for *A. viridis* irrespective of exposure to rhizo-inoculation (11.5 – 17.5 cm<sup>2</sup>,  $p=223$ ). No significant differences in number of leaves per plant, stem girth, and internode was reported in both species irrespective of treatment.

**Table 4.1:** Terminal field parameters at 65 days after sowing

	*Height (cm)	Leaf area (cm <sup>2</sup> )	*No. of leaves/ plant	Stem girth (cm)	length of leaf stalk (cm)	Internode (cm)
<b><i>Amaranthus hybridus</i></b>						
HMS Control	30.1 <sup>a</sup>	10.2 <sup>b</sup>	16 <sup>a</sup>	0.5 <sup>a</sup>	6.3 <sup>a</sup>	1.6 <sup>a</sup>
HMS( <i>P.f</i> )	33.3 <sup>a</sup>	15.2 <sup>ab</sup>	16 <sup>a</sup>	0.8 <sup>a</sup>	7.1 <sup>a</sup>	2.1 <sup>a</sup>
HMS( <i>P.p</i> )	31.1 <sup>a</sup>	21.3 <sup>a</sup>	19 <sup>a</sup>	0.6 <sup>a</sup>	6.8 <sup>a</sup>	1.8 <sup>a</sup>
HMS( <i>P.fp</i> )	39.2 <sup>a</sup>	17.3 <sup>a</sup>	18 <sup>a</sup>	0.8 <sup>a</sup>	6.4 <sup>a</sup>	1.5 <sup>a</sup>
p-value	0.293	0.046	0.135	0.327	0.097	0.306
<b><i>Amaranthus viridis</i></b>						
HMS Control	36.2 <sup>a</sup>	11.5 <sup>a</sup>	16 <sup>a</sup>	0.6 <sup>a</sup>	6.4 <sup>a</sup>	1.2 <sup>a</sup>
HMS( <i>P.f</i> )	41.6 <sup>a</sup>	16.3 <sup>a</sup>	19 <sup>a</sup>	0.8 <sup>a</sup>	7.3 <sup>a</sup>	1.5 <sup>a</sup>
HMS( <i>P.p</i> )	37.3 <sup>a</sup>	17.5 <sup>a</sup>	18 <sup>a</sup>	0.7 <sup>a</sup>	7.3 <sup>a</sup>	1.8 <sup>a</sup>
HMS( <i>P.fp</i> )	35.3 <sup>a</sup>	16.9 <sup>a</sup>	21 <sup>a</sup>	0.9 <sup>a</sup>	6.8 <sup>a</sup>	1.1 <sup>a</sup>
p-value	0.072	0.223	0.347	0.477	0.172	0.338

\*Presented in the nearest integers. Results show mean±SD

All plants in ferruginous soils died out before the the 40<sup>th</sup> day after sowing, attaining a maximum plant height of 6 cm

Below ground parameters of both plant species have been present on Table 4.2. In *A. hybridus*, primary root length significantly increased from 14.2 cm in the unprimed plant to 21.2 – 23.6 cm when same plant was rhizo-inoculated ( $p=0.011$ ). However, in *A. viridis*, rhizo-inoculation with the test bacteria did not significantly affect prominent root length ( $p>0.05$ ). Generally, however, rhizo-inoculation significantly enhanced root biomass by more than 60% in both plant species.

**Table 4.2:** Root growth parameters of seedling at 65 days after sowing

	Primary root length (cm)	Secondary root length (cm)	Root dry wt. (g)
<b><i>A. hybridus</i></b>			
HMS Control	14.2 <sup>b</sup>	10.4 <sup>a</sup>	0.59 <sup>b</sup>
HMS( <i>P.f</i> )	23.6 <sup>a</sup>	7.0 <sup>a</sup>	3.26 <sup>a</sup>
HMS( <i>P.p</i> )	21.2 <sup>ab</sup>	8.3 <sup>a</sup>	2.94 <sup>a</sup>
HMS( <i>P.fp</i> )	23.2 <sup>a</sup>	7.3 <sup>a</sup>	3.65 <sup>a</sup>
p-value	0.011	0.164	0.020
<b><i>A. viridis</i></b>			
HMS Control	15.6 <sup>a</sup>	9.6 <sup>a</sup>	0.62 <sup>b</sup>
HMS( <i>P.f</i> )	20.3 <sup>a</sup>	6.8 <sup>a</sup>	3.01 <sup>a</sup>
HMS( <i>P.p</i> )	19.9 <sup>a</sup>	7.4 <sup>a</sup>	3.95 <sup>a</sup>
HMS( <i>P.fp</i> )	21.7 <sup>a</sup>	6.4 <sup>a</sup>	4.01 <sup>a</sup>
p-value	0.093	0.321	0.007

Results show mean±SD

All plants in ferruginous soils died out before the the 40<sup>th</sup> day after sowing, attaining a maximum plant height of 6 cm

Maximum length of inflorescence as well as number of days required for flowering were both not significantly affected by rhizo-inoculation with the Pseudomonads ( $p>0.05$ ) (Table 4.3); whereas maximum inflorescence length was 10.2 – 13.7 cm in *A. hybridus* and 12.3 – 15.3 cm in *A. viridis*, the number of days required for flowering generally ranged from 46 – 53 days in both plant species.

**Table 4.3:** Flowering parameters of seedling at 65 days after sowing (means presented in whole numbers)

	<i>A. hybridus</i>		<i>A. viridis</i>	
	Max. length of inflorescence (cm)	Day to flowering (days)	Max. length of inflorescence (cm)	Day to flowering (days)
HMS Control	10.2 <sup>a</sup>	46 <sup>a</sup>	13.1 <sup>a</sup>	49 <sup>a</sup>
HMS( <i>P.f</i> )	11.4 <sup>a</sup>	48 <sup>a</sup>	16.6 <sup>a</sup>	44 <sup>a</sup>
HMS( <i>P.p</i> )	13.2 <sup>a</sup>	53 <sup>a</sup>	12.3 <sup>a</sup>	41 <sup>a</sup>
HMS( <i>P.fp</i> )	13.7 <sup>a</sup>	48 <sup>a</sup>	15.3 <sup>a</sup>	45 <sup>a</sup>
p-value	0.394	0.153	0.131	0.228

*NP - no plant. Results show mean ± SD*

In summary the rate of plant development measured for plant height, leaf area as well as time taken for single leaf formation has been presented on Table 4.4. This presentation was taken for the entire 65 days and as such the result taken for the ferruginous red soil treatments were omitted since the plant gave way between the 35 and the 45 days after sowing. Result showed that there were no significant differences in the rate of increase in plants height measured in cm per day (3.41-3.62cm/day) in *A. hybridus* and 2.98 – 3.28 cm/day in *A. viridis* respectively. For the rate of development of leave area, it was observed that for plant that were rhizo-inoculated by either *Pseudomonas putida* or *P. fluorescens* or a combination of both bacteria, the rate of development of leaf area ranged from 0.24 - 0.26cm<sup>2</sup> per day compared to 0.16 cm per day in the control for *A. hybridus*. Generally, it took significantly longer period for single leave formation in control plant (91.2 h in *A. hybridus* and 94.0 h in *A. viridis*), compared to their rhizo-inoculated counter parts (63.1 – 70.1 h in *A. hybridus* and 69.2 – 73.6 h in *A. viridis*).

**Table 4.4:** Rate of plant development

Parameters	Plant height (mm/d)	Leaf area (cm <sup>2</sup> /d)	Time per leaf formation (hr)
HMS Control	3.43 <sup>a</sup>	0.16 <sup>a</sup>	91.2 <sup>a</sup>
HMS( <i>P.f</i> )	3.41 <sup>a</sup>	0.25 <sup>a</sup>	68.5 <sup>b</sup>
HMS( <i>P.p</i> )	3.44 <sup>a</sup>	0.24 <sup>a</sup>	70.1 <sup>b</sup>
HMS( <i>P.fp</i> )	3.62 <sup>a</sup>	0.26 <sup>a</sup>	63.1 <sup>b</sup>
p-value	0.632	0.288	0.027
HMS Control	3.28 <sup>a</sup>	0.21 <sup>a</sup>	94.0 <sup>a</sup>
HMS( <i>P.f</i> )	3.07 <sup>a</sup>	0.18 <sup>a</sup>	71.2 <sup>b</sup>
HMS( <i>P.p</i> )	3.27 <sup>a</sup>	0.26 <sup>a</sup>	73.6 <sup>b</sup>
HMS( <i>P.fp</i> )	2.98 <sup>a</sup>	0.21 <sup>a</sup>	69.2 <sup>b</sup>
p-value	0.105	0.422	0.004

Means on the same column with similar alphabetic superscripts do not differ from each other ( $p > 0.05$ )

Plants sown in humus soil and rhizo-inoculated with *P. fluorescens* (HMS(*P.f*)), *P. putida* (HMS(*P.p*)), and a combination of both bacteria (HMS(*P.fp*)). Plants sown in red ferruginous soil and rhizo-inoculated with *P. fluorescens* (RFS(*P.f*)), *P. putida* (RFS(*P.p*)), and a combination of both bacteria (RFS(*P.fp*)).

Table 4.5 shows soil nutrient parameters before and after sowing. After removal of *A. hybridus* plants, total organic carbon (TOC) in the red ferruginous soils whether rhizo-inoculated or not ranged from 0.49 to 0.68% compare to that in the humus soil, irrespective of rhizo-inoculation 2.69-2.96%. For those soils with *A. viridis*, TOC in ferruginous soils was 0.4 – 0.64%. Generally, more than 45% decreases in concentrations of N, P and K were reported in the ferruginous soils compared to their humus counterparts. Being ferruginous in nature, the ferruginous red soils generally had significant increased iron content 442.34 – 640.88 mg/kg, compared to a range of 31.44 – 79.30 mg/kg of iron in the humus soil.

**Table 4.5:** Soil nutrient parameters before and after sowing

	TOC	N	P	K	Fe
	(%)		(mg/kg)		
HMS (at start)	3.83	3.003	2.048	1.96	72.55
RFS (at start)	0.102	2.024	0.103	0.83	801.2
HMS Control	2.83 <sup>a</sup>	2.102 <sup>a</sup>	0.148 <sup>a</sup>	1.23 <sup>a</sup>	60.55 <sup>b</sup>
HMS( <i>P.f</i> )	2.82 <sup>a</sup>	2.078 <sup>a</sup>	0.178 <sup>a</sup>	1.25 <sup>a</sup>	56.32 <sup>b</sup>
HMS( <i>P.p</i> )	2.96 <sup>a</sup>	2.079 <sup>a</sup>	0.166 <sup>a</sup>	1.48 <sup>a</sup>	48.24 <sup>b</sup>
HMS( <i>P.fp</i> )	2.69 <sup>a</sup>	2.753 <sup>a</sup>	0.183 <sup>a</sup>	1.38 <sup>a</sup>	52.54 <sup>b</sup>
RFS Control	0.49 <sup>b</sup>	1.447 <sup>b</sup>	0.089 <sup>b</sup>	0.59 <sup>b</sup>	619.3 <sup>a</sup>
RFS( <i>P.f</i> )	0.68 <sup>b</sup>	1.314 <sup>b</sup>	0.058 <sup>b</sup>	0.48 <sup>b</sup>	501.2 <sup>a</sup>
RFS( <i>P.p</i> )	0.56 <sup>b</sup>	1.045 <sup>b</sup>	0.072 <sup>b</sup>	0.49 <sup>b</sup>	581.2 <sup>a</sup>
RFS( <i>P.fp</i> )	0.62 <sup>b</sup>	1.182 <sup>b</sup>	0.097 <sup>b</sup>	0.95 <sup>b</sup>	601.2 <sup>a</sup>
p-value	0.024	0.003	0.032	0.011	<0.001
HMS Control	2.93 <sup>ab</sup>	2.182 <sup>a</sup>	0.153 <sup>ab</sup>	1.27 <sup>a</sup>	62.66 <sup>b</sup>
HMS( <i>P.f</i> )	2.28 <sup>ab</sup>	2.015 <sup>a</sup>	0.185 <sup>ab</sup>	1.29 <sup>a</sup>	79.30 <sup>b</sup>
HMS( <i>P.p</i> )	3.85 <sup>a</sup>	2.081 <sup>a</sup>	0.171 <sup>ab</sup>	1.53 <sup>a</sup>	31.44 <sup>b</sup>
HMS( <i>P.fp</i> )	1.79 <sup>b</sup>	2.153 <sup>a</sup>	0.196 <sup>ab</sup>	1.43 <sup>a</sup>	89.24 <sup>b</sup>
RFS Control	0.51 <sup>c</sup>	1.754 <sup>a</sup>	0.099 <sup>ab</sup>	0.61 <sup>b</sup>	640.88 <sup>a</sup>
RFS( <i>P.f</i> )	0.40 <sup>c</sup>	1.977 <sup>a</sup>	0.083 <sup>b</sup>	0.50 <sup>b</sup>	442.34 <sup>a</sup>
RFS( <i>P.p</i> )	0.58 <sup>c</sup>	2.081 <sup>a</sup>	0.071 <sup>b</sup>	0.51 <sup>b</sup>	601.46 <sup>a</sup>
RFS( <i>P.fp</i> )	0.64 <sup>c</sup>	1.892 <sup>a</sup>	0.101 <sup>ab</sup>	0.98 <sup>ab</sup>	502.24 <sup>a</sup>
p-value	0.046	0.101	0.054	0.036	<0.001

Means on the same column with similar alphabetic superscripts do not differ from each other ( $p > 0.05$ )  
 TOC total organic carbon, TN total nitrogen, P total phosphate, K potassium, Fe iron

Plants sown in humus soil and rhizo-inoculated with *P. fluorescens* (HMS(*P.f*)), *P. putida* (HMS(*P.p*)), and a combination of both bacteria (HMS(*P.fp*)). Plants sown in red ferruginous soil and rhizo-inoculated with *P. fluorescens* (RFS(*P.f*)), *P. putida* (RFS(*P.p*)), and a combination of both bacteria (RFS(*P.fp*)).

Plate 4.1a and b show plant species in both ferruginous red soils and humus soils (black in colour) after rhizoinoculation.



(a)



(b)

**Plate 4.1a and b:** Presentation of plant species in both ferruginous red soils and humus soils (black in colour) after rhizoinoculation

Plate 4.2 shows *Amaranthus hybridus* in the red ferruginous soil inoculated with *Pseudomonas putida* (Plate 4.2a) as well as that inoculated with *P. fluorescens* (Plate 4.2b) and sown in humus and red soils respectively after 22 days after sowing. Plate 4.2a, Plate 4.2b, Plate 4.2c (R), and Plate 4.3a show 1-cm tall amaranths already chlorotic with brown to purple stems. These plants died out before the 45<sup>th</sup> day after sowing (Plate 4.3b).



(a)

(b)



(c)

**Plate 4.2a, b and c:** *Amaranthus hybridus* in (a) red soil inoculated with *Pseudomonas putida* (b) red soil inoculated with *P. fluorescens* (c) humus and red soils respectively upon inoculation with *P. fluorescens* at 22 days after sowing



(a)



(b)

**Plate 4.3a and b:** *P. Fluorescens* inoculated *Amaranthus hybridus* in both humus and red soils after (a) 22 days (b) 45 days following sowing (note visible plants after 45 days in red soil)

Plate 4.4a compares plant morphological presentation of test plant sown in humus soil at 40 days after sowing and exposure to test organisms. Plate 4.4b shows the plants exposed to *P. fluorescens*.

**Plate 4.4c:** Inflorescence of *Amaranthus hybridus* under influence of *P. fluorescens* compared to the control. The qualitative assessment of glycosides, saponins, phenolic, tannins, steroids, terpenoids, alkaloids, and flavonoids in both plant species after exposure to inoculating bacteria have been presented (Table 4.6). Saponins, phenolic, tannins, terpenoids, alkaloids, and flavonoids were present in both species notwithstanding whether the plants were inoculated or not. The presence of glycosides in plants inoculated with *P. pseudomonas* was reported. Glycosides were absent in the control plants.



(a)



(b)



(c)

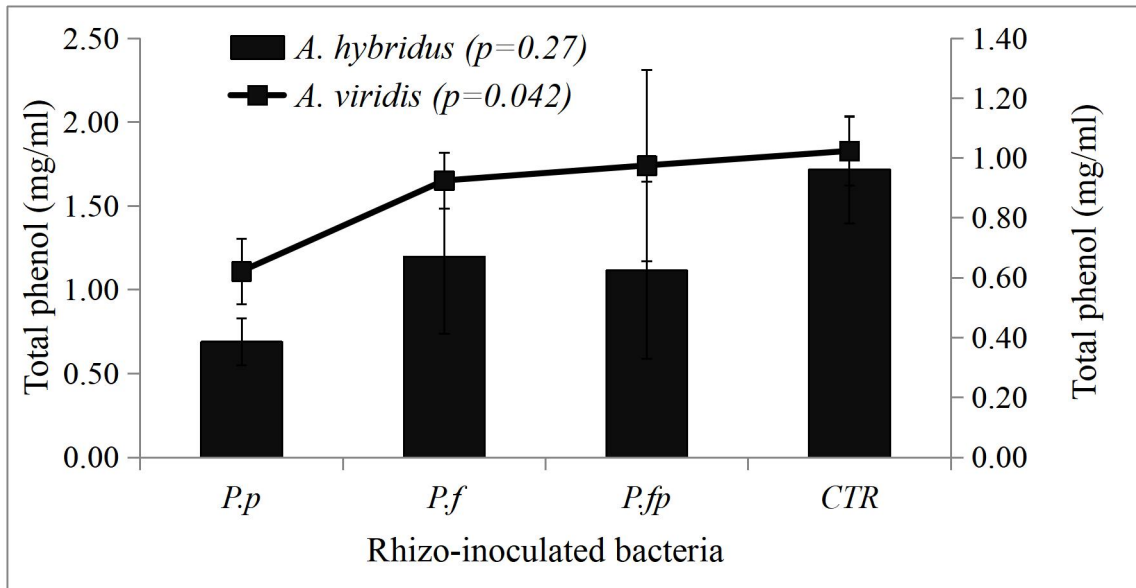
**Plate 4.4a: Plant morphological presentation of test plant sown in humus soil at 40 days after sowing and exposure to test organisms. H(B) Plants exposed in humus filtrates and inoculated with both bacteria, *Pseudomonas putida* H(P), *P. fluorescens* H(F) for *Amaranthus hybridus*, whereas their respective counterparts in *A. viridis* were V(B), V(P) and V(F) respectively.**

**Table 4.6:** Phytochemical analysis of analysis *Amaranthus hybridus* and *Amaranthus viridis*

	<i>Amaranthus hybridus</i>				<i>Amaranthus viridis</i>			
	HMS ( <i>P.p</i> )	HMS ( <i>P.f</i> )	HMS ( <i>P.fj</i> )	HMS Control	HMS ( <i>P.p</i> )	HMS ( <i>P.f</i> )	HMS ( <i>P.fj</i> )	HMS Control
Glycosides	+	-	-	-	+	-	+	-
Saponins	++	++	++	+	++	++	+	++
Phenolics	++	++	+	++	++	+	+	++
Tannins	+	+	+	+	+	+	+	++
Steroids	-	+	+	++	-	-	+	+
Terpenoids	++	++	++	++	++	++	++	++
Alkaloids	+	+	+	+	+	++	++	+
Flavonoids	++	++	+	++	++	++	++	++

Key: Degree of presence based on qualitative colour changes (++, +), - negative

There was significant reduction in total phenols upon inoculation of plants with *P. putida* (Fig. 4.1). Total phenols in *A. hybridus* inoculated with *P. putida* was 0.690 mg/ml compared to 1.717 mg/ml in the control. Similarly, a reduction in phenols from 1.023 mg/ml to 0.621 mg/ml was reported in *A. viridis*. However, no significant changes in total phenols was reported with both plants were rhizo-inoculated with a combination of both Pseudomonads.



**Fig. 4.1:** Total phenolic content of *A. hybridus* and *A. viridis* using Jenway UV Spectrophotometer (model 6715) at 760nm wavelength.

Table 4.7 shows the survival time of test plants under experimental conditions on the field. Survival time was beyond 65 days in the control for both plant species. These plants showed Good growth and green leaves. Less than 33% leaves/plant showed chlorotic signs. Plant height was above 30 cm. Plant species inoculated with test bacteria, but sown in humus soil, also had a survival time of beyond 65 days. For these plant species, good growth, leaves were green, with only less than 20% showing chlorotic signs. Plant height within this category was above 30 cm, same as control. Notwithstanding the type of bacterial species used for rhizoinoculation, plant species in ferruginous red soils died out within 21 – 35 days after exposure. Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves (brownish) produced was 6 with combined leaf area of less than 3cm<sup>2</sup>.

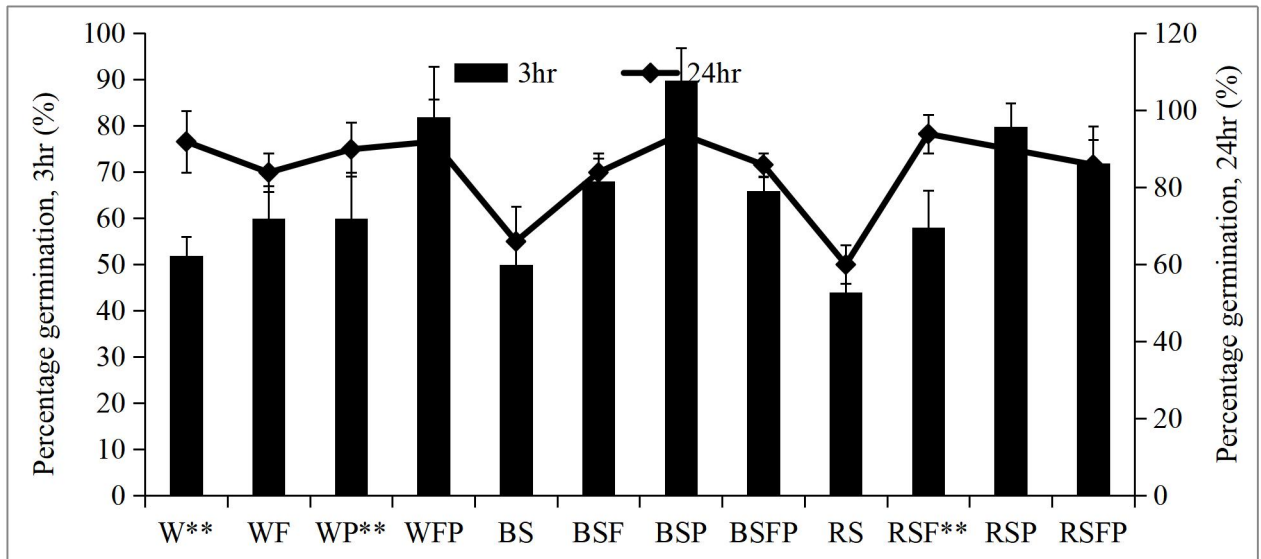
**Table 4.7:** Survival time of test plants under experimental conditions on the field

	<i>A. hybridus</i>		<i>A. viridis</i>	
	*Survival time (d)	Plant observation for 65 days	Survival time (d)	Plant observation for 65 days
HMS Control	> 65	Good growth. Leaves green. Less than 33% leaves/plant showed chlorotic signs. Plant height above 30 cm.	> 65	Good growth. Leaves green. Less than 25% leaves/plant showed chlorotic signs. Plant height above 30 cm.
HMS( <i>P.f</i> )	> 65	Good growth. Leaves green. Less than 20% leaves/plant showed chlorotic signs. Plant height above 30 cm, same as control.	> 65	Good growth. Leaves green. Less than 20% leaves/plant showed chlorotic signs. Plant height above 30 cm, same as control.
HMS( <i>P.p</i> )	> 65	Good growth. Leaves green. Less than 15% leaves showed chlorotic signs. Plant height above 30 cm, same as control.	> 65	Good growth. Leaves green. Less than 20% leaves showed chlorotic signs. Plant height above 30 cm, same as control.
HMS( <i>P.fp</i> )	> 65	Good growth. Leaves green. Less than 10% showed chlorotic signs. Plants have more leaves than control.	> 65	Good growth. Leaves green. Less than 10% showed chlorotic signs. Plants have more leaves than control.
RFS Control	21±6	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves (brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>	25±6	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 5 with combined leaf area of less than 3cm <sup>2</sup>
RFS( <i>P.f</i> )	29±4	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>	31±4	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 5 with combined leaf area of less than 3cm <sup>2</sup>
RFS( <i>P.p</i> )	31±5	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>	33±5	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>
RFS( <i>P.fp</i> )	35±5	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>	41±2	Maximum plant height attained was < 2cm. Plant stem was reddish brown. Average maximum number of leaves(brownish) produced was 6 with combined leaf area of less than 3cm <sup>2</sup>

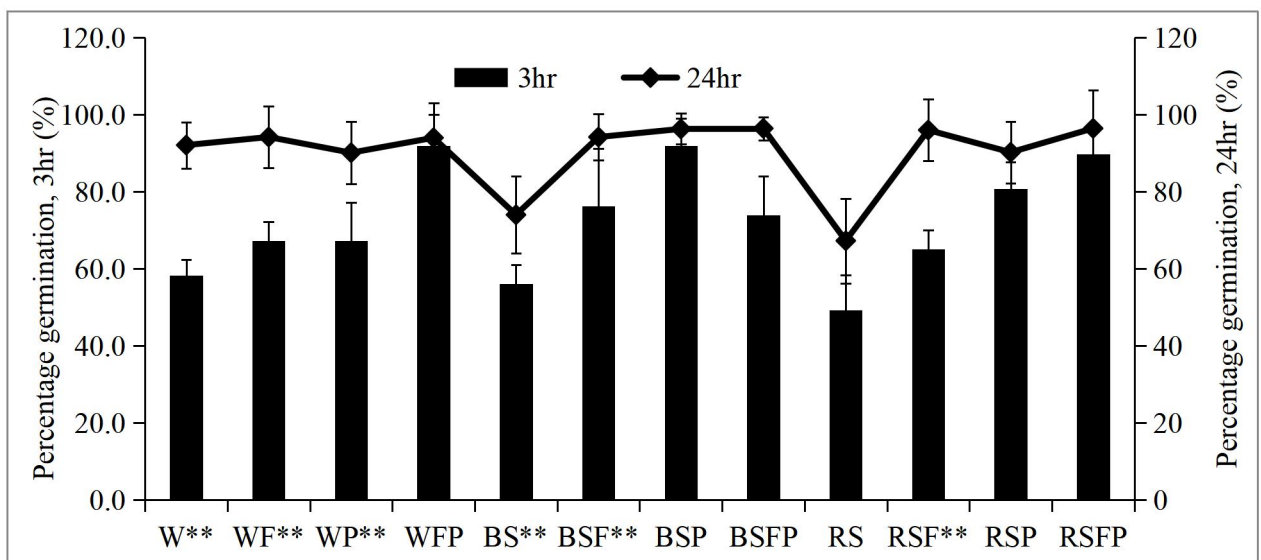
\*Experiment was terminated on 65<sup>th</sup> day. Results presented in the nearest integers. Results show mean±SD

## 4.2 Germination Studies

In *A. hybridus*, germination percentage after 9 days following germination initiation and exposure to test microorganisms showed significant differences in germination percentage when seeds were bio-primed with test organisms for 3 hours and 24 hours respectively, particularly for WP and RSP (Fig. 2a). Similarly, it was observed that seeds that were presoaked in distilled water (W) before sowing in petri dishes had a better germination percentage (91.8%) when they were presoaked for 24 hours than when they were for 3 hours (51.9%). Generally, germination percentage for *Pseudomonas*-exposed seeds was generally better than with the longer exposure time than in the 3-hours exposure time. Similar germination patterns were reported in *A. viridis* (Fig. 4.2b).



**Fig. 4.2(a):** Germination percentage of *A. hybridus* at 9 days after germination initiation (\*\* significant at  $p < 0.05$ )



**Fig. 4.2(b):** Germination percentage of *A. viridis* at 9 days after germination initiation (\*\* significant at  $p < 0.05$ )

Means on the same column with similar alphabetic superscripts do not differ from each other ( $p > 0.05$ )

(W) were seeds primed in distilled water and sown in petri dishes moistened with distilled water. Other seeds sown in petri dishes with distilled water were primed in *P. fluorescens* (WF), *P. putida* (WP) and a combination of both bacteria (WFP). Seeds sown in petri dishes moistened with filtrates from humus soil were primed in distilled water (BS), *P. fluorescens* (BSF), *P. putida* (BSP) and a combination of both bacteria (BSFP). Other seeds sown were moistened with filtrates from red fruginous soil and primed in distilled water (RS), *P. fluorescens* (RSF), *P. putida* (RSP) and a combination of both bacteria (RSFP).

Results of selected growth parameters of the germinant taken at 9 days after initiation of germination have been presented on Table 4.8. Result showed that there were no significant differences in leaf length, shoot length as well as root length in both plant species irrespective of the time of bio-priming or the material or micro-organism used for the priming. In this case leaf length generally ranged from 2.8mm to 3.8mm ( $p>0.05$ ). Root length therefore ranged from 11mm in *Amaranthus hybridus* primed in water for 24 hours and sown in petri dishes with distilled water as medium to 21.7mm in *Amaranthus hybridus* bio-primed for 24hours in *P. pseudomonas* and sown in petri dishes with distilled water (Table 4.8). With respect to effects of time priming, seeds at 3h and those primed at 24h did not show significant differences in measured parameters for *A. hybridus*. However, in *A. viridis*, root length was higher in those plants primed with *P. fluorescens* for 24 h in distilled water (22.5 mm) was higher than those primed for 3 h (17.6 mm).

**Table 4.8:** Growth parameters of *A. hybridus* and *A. viridis* seedlings at 9 days after initiation

	leaf length(mm)			shoot length (mm)			root length (mm)		
	24hr	3hr	P	24hr	3hr	p	24hr	3hr	P
<b><i>Amaranthus hybridus</i></b>									
W	3.0 <sup>a</sup>	3.0 <sup>a</sup>	0.623	20.0 <sup>a</sup>	23.7 <sup>a</sup>	0.142	11.0 <sup>a</sup>	17.3 <sup>a</sup>	0.426
WF	3.1 <sup>a</sup>	3.1 <sup>a</sup>	0.311	28.0 <sup>a</sup>	25.0 <sup>a</sup>	0.213	21.7 <sup>a</sup>	16.7 <sup>a</sup>	0.346
WP	3.0 <sup>a</sup>	3.0 <sup>a</sup>	0.632	23.0 <sup>a</sup>	24.7 <sup>a</sup>	0.104	15.0 <sup>a</sup>	16.3 <sup>a</sup>	0.135
WFP	3.0 <sup>a</sup>	3.0 <sup>a</sup>	0.244	22.7 <sup>a</sup>	21.7 <sup>a</sup>	0.311	18.7 <sup>a</sup>	19.3 <sup>a</sup>	0.425
BS	3.1 <sup>a</sup>	3.0 <sup>a</sup>	0.692	30.3 <sup>a</sup>	28.7 <sup>a</sup>	0.487	20.3 <sup>a</sup>	13.3 <sup>a</sup>	0.291
BSF	3.2 <sup>a</sup>	3.3 <sup>a</sup>	0.553	25.3 <sup>a</sup>	23.7 <sup>a</sup>	0.129	13.3 <sup>a</sup>	17.3 <sup>a</sup>	0.312
BSP	3.3 <sup>a</sup>	3.2 <sup>a</sup>	0.272	23.7 <sup>a</sup>	22.7 <sup>a</sup>	0.503	15.3 <sup>a</sup>	18.3 <sup>a</sup>	0.138
BSFP	3.1 <sup>a</sup>	3.2 <sup>a</sup>	0.400	21.3 <sup>a</sup>	24.0 <sup>a</sup>	0.329	17.7 <sup>a</sup>	19.3 <sup>a</sup>	0.481
RS	3.1 <sup>a</sup>	2.8 <sup>a</sup>	0.634	23.0 <sup>a</sup>	22.0 <sup>a</sup>	0.359	14.7 <sup>a</sup>	16.0 <sup>a</sup>	0.423
RSF	3.1 <sup>a</sup>	3.1 <sup>a</sup>	0.113	26.7 <sup>a</sup>	21.3 <sup>a</sup>	0.135	18.0 <sup>a</sup>	16.0 <sup>a</sup>	0.291
RSP	3.3 <sup>a</sup>	3.0 <sup>a</sup>	0.214	26.0 <sup>a</sup>	21.3 <sup>a</sup>	0.094	15.0 <sup>a</sup>	17.7 <sup>a</sup>	0.309
RSFP	3.3 <sup>a</sup>	3.1 <sup>a</sup>	0.577	22.0 <sup>a</sup>	18.0 <sup>a</sup>	0.103	19.0 <sup>a</sup>	20.7 <sup>a</sup>	0.261
p-value	0.138	0.237	-	0.624	0.423	-	0.141	0.105	-
<b><i>Amaranthus viridis</i></b>									
W	3.3 <sup>a</sup>	3.0 <sup>a</sup>	0.742	20.8 <sup>a</sup>	24.5 <sup>a</sup>	0.333	14.3 <sup>a</sup>	17.6 <sup>a</sup>	0.054
WF	3.2 <sup>a</sup>	3.0 <sup>a</sup>	0.423	28.9 <sup>a</sup>	25.8 <sup>a</sup>	0.126	22.5 <sup>a</sup>	19.7 <sup>a</sup>	<b>0.023</b>
WP	3.3 <sup>a</sup>	3.1 <sup>a</sup>	0.662	24.4 <sup>a</sup>	25.8 <sup>a</sup>	0.162	15.4 <sup>a</sup>	16.6 <sup>a</sup>	0.148
WFP	2.9 <sup>a</sup>	3.0 <sup>a</sup>	0.137	22.6 <sup>a</sup>	22.4 <sup>a</sup>	0.387	19.6 <sup>a</sup>	19.7 <sup>a</sup>	0.336
BS	3.3 <sup>a</sup>	3.2 <sup>a</sup>	0.482	28.8 <sup>a</sup>	30.4 <sup>a</sup>	0.137	20.9 <sup>a</sup>	18.6 <sup>a</sup>	0.166
BSF	3.5 <sup>a</sup>	3.2 <sup>a</sup>	0.348	25.8 <sup>a</sup>	24.5 <sup>a</sup>	0.319	13.2 <sup>a</sup>	17.6 <sup>a</sup>	0.124
BSP	3.6 <sup>a</sup>	3.3 <sup>a</sup>	0.746	23.9 <sup>a</sup>	23.4 <sup>a</sup>	0.183	15.7 <sup>a</sup>	18.7 <sup>a</sup>	0.141
BSFP	3.2 <sup>a</sup>	3.0 <sup>a</sup>	0.649	21.6 <sup>a</sup>	25.8 <sup>a</sup>	<b>0.044</b>	18.6 <sup>a</sup>	19.7 <sup>a</sup>	0.491
RS	3.0 <sup>a</sup>	3.1 <sup>a</sup>	0.664	24.3 <sup>a</sup>	23.7 <sup>a</sup>	0.413	15.5 <sup>a</sup>	16.3 <sup>a</sup>	0.301
RSF	3.6 <sup>a</sup>	3.0 <sup>a</sup>	0.304	25.8 <sup>a</sup>	22.0 <sup>a</sup>	0.201	18.1 <sup>a</sup>	16.3 <sup>a</sup>	0.297
RSP	3.5 <sup>a</sup>	3.2 <sup>a</sup>	0.428	27.2 <sup>a</sup>	22.7 <sup>a</sup>	0.118	15.8 <sup>a</sup>	18.1 <sup>a</sup>	0.315
RSFP	3.8 <sup>a</sup>	3.3 <sup>a</sup>	0.329	23.7 <sup>a</sup>	22.7 <sup>a</sup>	0.176	19.2 <sup>a</sup>	21.1 <sup>a</sup>	0.206
p-value	0.236	0.302	-	0.129	0.302	-	0.062	0.332	-

Means on the same column with similar alphabetic superscripts do not differ from each other ( $p > 0.05$ )

The weight of *Amaranthus hybridus* seedlings taken at 9 days after initiation of germination have been presented on Table 4.9. Results showed significant differences in 20 germinant weight for test plant for bio-primed in *P. putida* for 24hours (1.10g) than that which was bio-primed in *P. putida* for 3h (0.12g) and sown in petri dishes with humus soil filtrate ( $p < 0.001$ ). There were also significant differences in 20 germinant weight for in the priming time for *A. hybridus* bio-primed in *P. putida* and sown in red soil filtrate as well as ( $p = 0.037$ ) as well as in a combination of both *P. fluorescens* and *P. putida* sown in petri dishes with red soil filtrate  $p = 0.041$ .

For *A. viridis*, significant differences in weight between those seeds primed in *P. putida* in humus soil filtrate was also reported. Lengthened priming time also significantly increased weights of germinants for seeds primed in the red soil filtrates.

**Table 4.9:** Weight of amaranth seedlings at 9 days after initiation

	20 germinant weight*					
	<i>A. hybridus</i>			<i>A. viridis</i>		
	24hr	3hr	p	24hr	3hr	P
W	0.01 <sup>c</sup>	0.04 <sup>b</sup>	0.118	0.01 <sup>c</sup>	0.10 <sup>b</sup>	<b>0.002</b>
WF	0.10 <sup>d</sup>	0.06 <sup>b</sup>	0.346	0.20 <sup>c</sup>	0.10 <sup>b</sup>	0.082
WP	0.08 <sup>d</sup>	0.09 <sup>b</sup>	0.132	0.27 <sup>b</sup>	0.20 <sup>a</sup>	0.642
WFP	0.90 <sup>ab</sup>	0.11 <sup>b</sup>	0.227	0.19 <sup>c</sup>	0.11 <sup>ab</sup>	0.392
BS	0.12 <sup>d</sup>	0.10 <sup>b</sup>	0.128	0.12 <sup>c</sup>	0.11 <sup>ab</sup>	0.128
BSF	0.07 <sup>d</sup>	0.05 <sup>b</sup>	0.319	0.20 <sup>c</sup>	0.21 <sup>a</sup>	0.336
BSP	1.10 <sup>a</sup>	0.12 <sup>b</sup>	<b>&lt;0.001</b>	0.57 <sup>ab</sup>	0.12 <sup>ab</sup>	<b>&lt;0.001</b>
BSFP	0.07 <sup>d</sup>	0.09 <sup>b</sup>	0.237	0.13 <sup>c</sup>	0.09 <sup>b</sup>	0.201
RS	0.80 <sup>bc</sup>	0.10 <sup>b</sup>	0.164	0.18 <sup>c</sup>	0.10 <sup>b</sup>	<b>0.034</b>
RSF	0.10 <sup>d</sup>	0.08 <sup>b</sup>	0.394	0.27 <sup>b</sup>	0.08 <sup>b</sup>	<b>0.009</b>
RSP	0.70 <sup>c</sup>	0.07 <sup>b</sup>	<b>0.037</b>	0.70 <sup>a</sup>	0.08 <sup>b</sup>	<b>&lt;0.001</b>
RSFP	0.10 <sup>d</sup>	0.80 <sup>a</sup>	<b>0.041</b>	0.48 <sup>ab</sup>	0.21 <sup>a</sup>	<b>0.038</b>
p-value	<0.001	0.017	-	0.004	0.022	-

\*Germinants were airdried for 5 hrs. Means on the same column with similar alphabetic superscripts do not differ from each other ( $p>0.05$ )

Plate 4.5 shows seeds of (a) *A. hybridus* and (b) *A. viridis* in Petri dishes on the day of sowing. Plate 4.5 shows the germinant as exposed to a combination of *P. fluorescens* and *P. putida* in distilled water (Plate 4.5a), *P. fluorescens* in water (Plate 5b) *P. fluorescens* in humus filtrate (Plate 4.5c), *P. fluorescens* in red soil filtrate at both 24 hours and 3 hours after exposure to test bacterial (Plate 4.5d). It should be noted that germinant in Plate 4.5d were already showing signs of lodging.

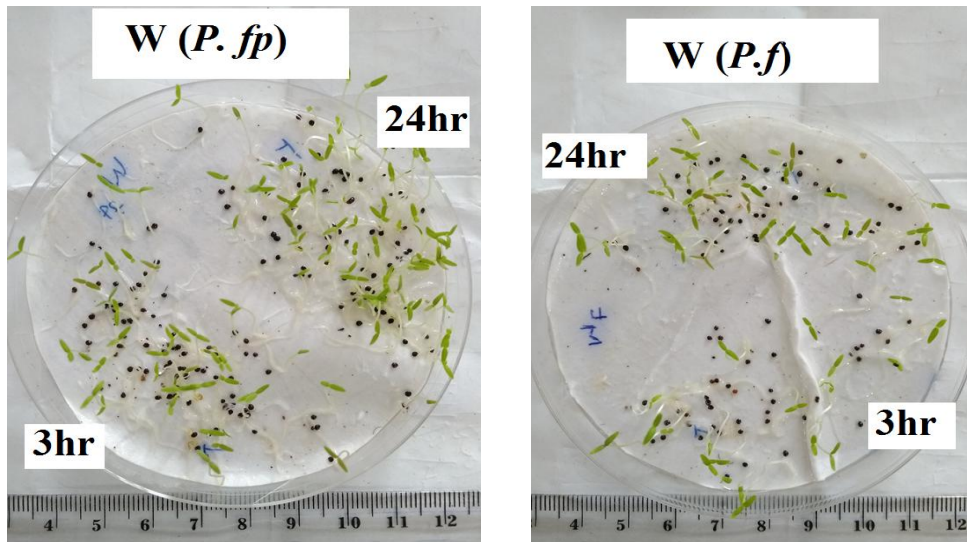


(a)

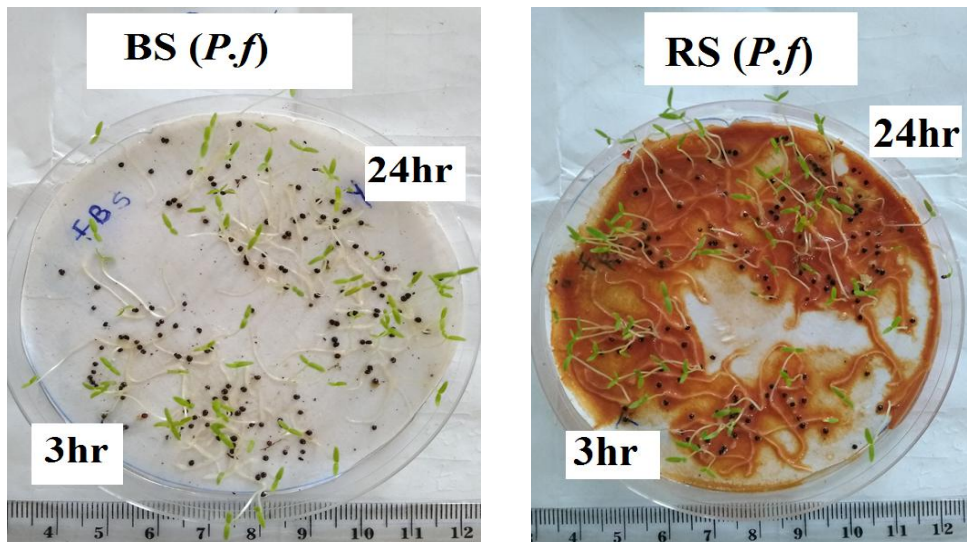


(b)

**Plate 4.5:** Seeds of (a) *A. hybridus* and (b) *A. viridis* in Petri dishes on the day of sowing



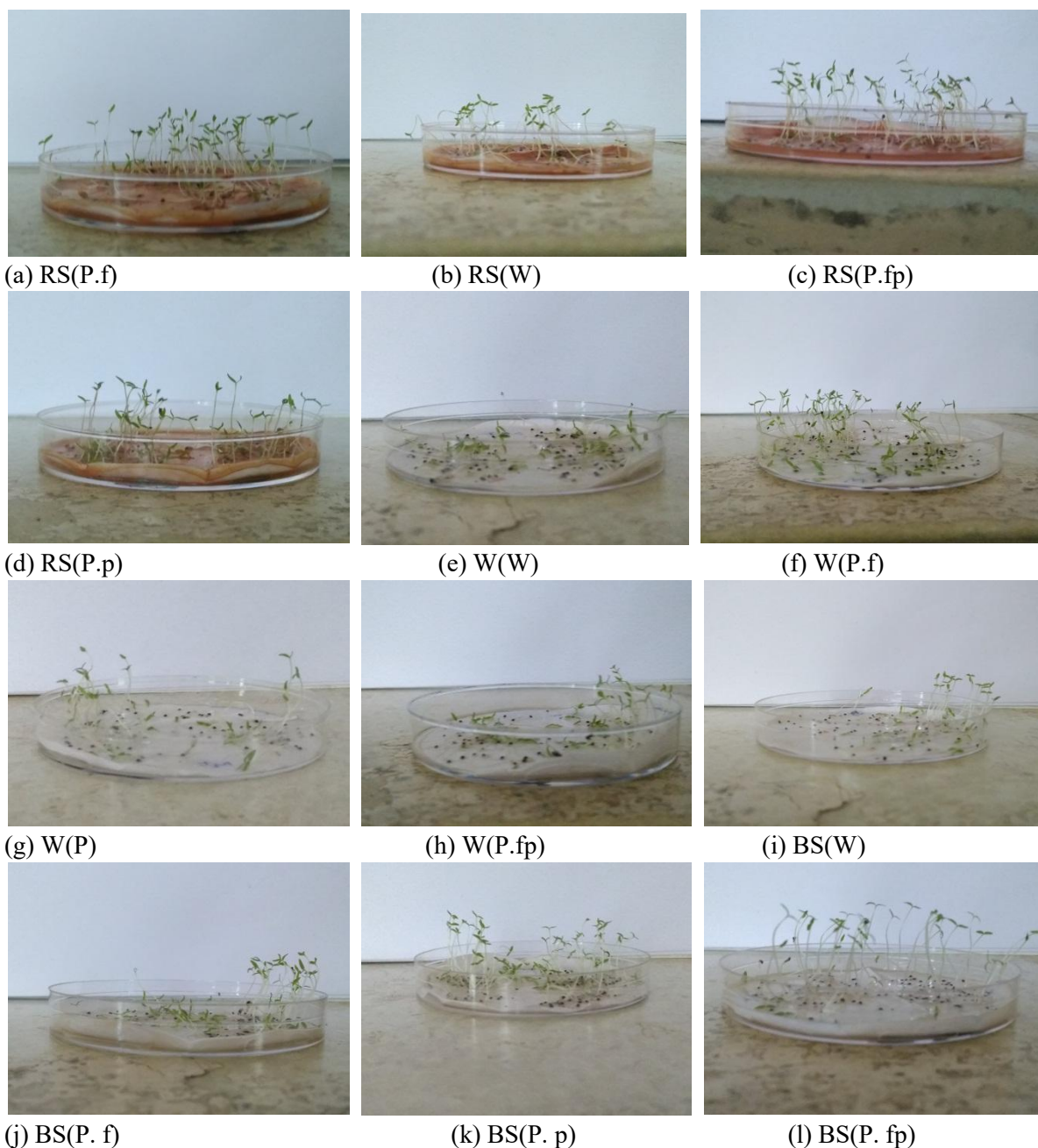
(a)



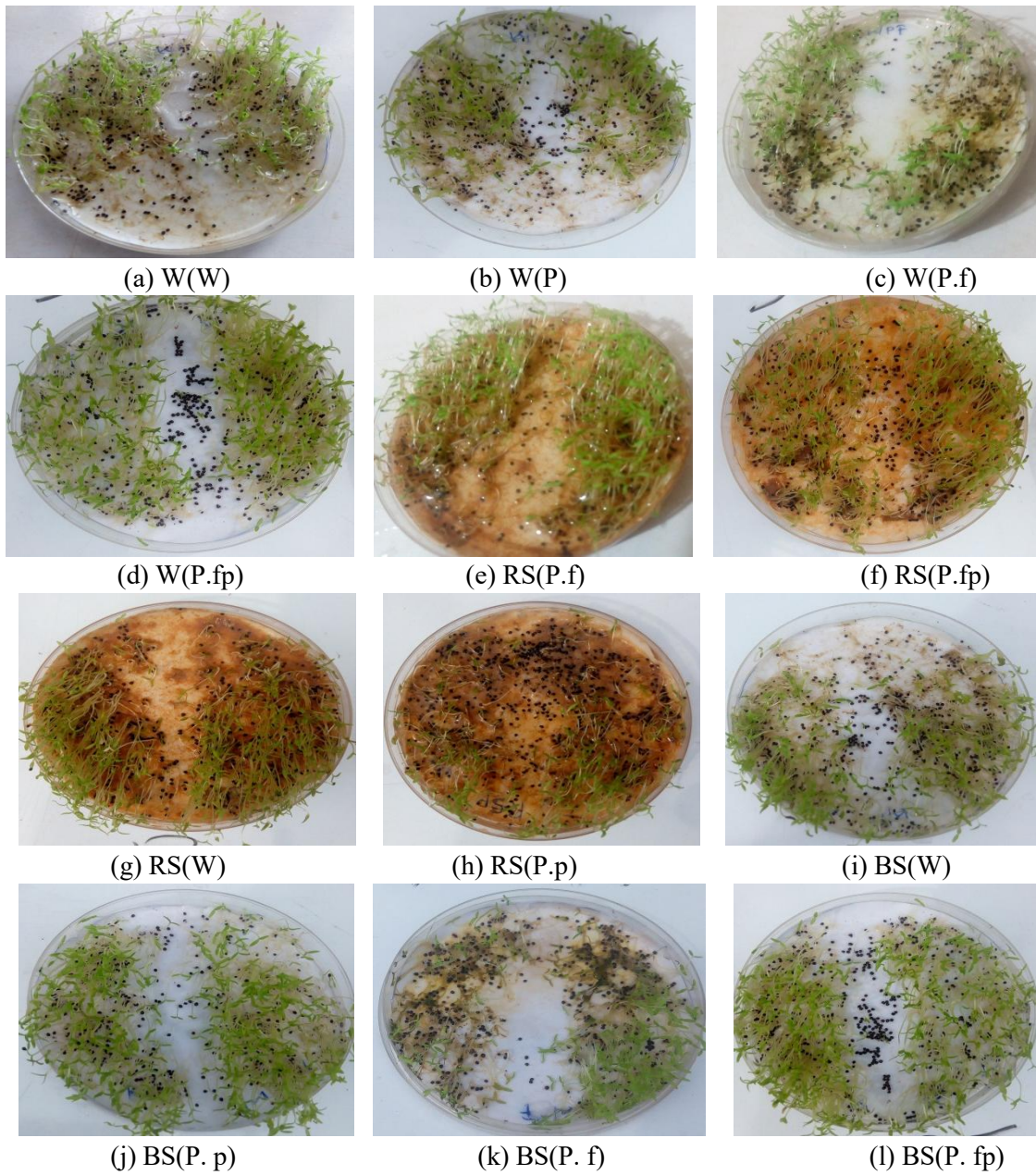
(b)

**Plate 4.6:** presentation of germinant exposed to (a) *Pseudomonas fluorescens* and *P. putida* in water (b) *Pseudomonas fluorescens* in water (c) *Pseudomonas fluorescens* in humus soil filtrate (d) *Pseudomonas fluorescens* in red soil filtrate in both 3 hours and 5 hours after exposure to test bacteria

Plates 4.7 and 4.8 present the germinant in petri dishes at 9 days after initiation of germination. As presented the plate showed *A. hybridus* and *A. viridis* germinants bio-primed with *P. fluorescens* and sown in petri dishes moistened with red soil filtrate (Plate 4.7a and Plate 4.8a respectively), followed by germinant primed in water and sown in red soil filtrate (Plate 4.7b, 4.8b), germinant bio-primed with a combination of both *P. fluorescens* and *P. putida* and sown in petri dishes moistened with red soil filtrate (Plate 4.7c, 4.8c), germinant bio-primed with *P. putida* and sown in petri dishes moistened with red soil filtrate (Plate 4.7d, 4.8d), germinant primed in water and sown in petri dishes moistened with water (Plate 4.7e, 4.8e), germinant bio-primed in *P. fluorescens* and sown in petri dishes moistened with water (Plate 4.7f and 4.8f), germinant bio-primed with *P. putida* and sown in petri dishes moistened with water (Plate 4.7g and 4.8g), germinant bio-primed with a combination of both *P. fluorescens* and *P. putida* and sown in petri dishes moistened with water (Plate 4.7h, 4.8h), germinant primed in water and sown in petri dishes moistened with humus soil filtrate (Plate 4.7i, 4.8i), germinant bio-primed with *P. fluorescens* and sown in petri dishes moistened with humus soil filtrate (Plate 4.7j and 4.8j), germinant bio-primed with *P. putida* and sown in petri dishes moistened with humus soil filtrate (Plate 4.7k and 4.8k) and germinant bio-primed with a combination of both *P. fluorescens* and *P. putida* and sown in petri dishes moistened with humus soil filtrate (Plate 4.7l and 4.8l).



**Plate 4.7:** *Amaranthus hybridus* germinants in petri dishes at 9 days after initiation of germination. RS – filtrates from red soil, BS – filtrates from humus soil, W – distilled water, (P.p) – added inoculum of *Pseudomonas putida*, (P.f) – added inoculum of *Pseudomonas fluorescens*, (P.fp) – added inoculum of both *P. putida* and *P. fluorescens*, (W) – no Pseudomonad, but distilled water.



**Plate 4.8:** *Amaranthus viridis* germinants in petri dishes at 9 days after initiation of germination. RS – filtrates from red soil, BS – filtrates from humus soil, W – distilled water, (P.p) – added inoculum of *Pseudomonas putida*, (P.f) – added inoculum of *Pseudomonas fluorescens*, (P.fp) – added inoculum of both *P. putida* and *P. fluorescens*, (W) – no Pseudomonad, but distilled water.

## CHAPTER FIVE

### 5.0

### DISCUSSION

A lot of works has been done to prove the potency of PGPRs especially the genus of *Pseudomonas* to promote growth of plant in stress conditions. This study has however shifted focus to suit the situation of the Edo state soil with is rich in iron oxide the reason for the redness of the soil and also comparing with the same treatment in humus soil (Imasuen and Onyeobi, 2013). At the end of the field experiment (after 65 days), It was however, observed that of all the treatment for both amaranthus species, those done on red soil (ferruginous soils) had zero readings because they all died off before the termination of the experiment at the 65<sup>th</sup> day after initiation, this is however irrespective of treatment with test the organisms. Before the *Amaranthus* plant gave way, they suffered great retardation in growth as they did not grow beyond the height of 1.6cm for *A. hybridus* and 1.9cm for *A. viridis* even after 25 days, a great retardation indeed when compared to minimum height of 7.7cm in the equivalent treatment in the humus soil. This is also the same in the case of number of leaves where the plants in the red soil had a maximum of 5 leaves for *A. hybridus* and 7 leaves for *A. viridis* on the same day in which the plants in the humus soil had a minimum and a maximum of 8 and 11 respectively.

### 5.2 Influence of ferruginicity

This great retardation can be related to the toxic effect of iron when it is in excess concentration in the soil as reported by Rasken *et al.*, 2000. As reported, it could either be as a result of the excess iron impairing the absorption of other nutrient which are necessary for the proper growth of the plant such as magnesium, potassium, phosphorus, calcium, among others. This is even more evident as the plants were never green even on germination till, they gave way (chlorosis from germination till death), an implication that the absorption of

magnesium could have been hindered. Another mechanism could have been the induction of oxidative stress in the plant which finally killed the plant (mami 2011).

However, when the time of death of the plants in the red soil was compared, plants treated with the *Pseudomonas spp.* took longer time to die, an indication that there was a level of stress tolerance induced in the treated plant which made them live longer than the control. From the report the plants treated with the combination of *Pseudomonas fluorescens* and *P. putida* had the longest life as it lived up to 45 days after the initiation of the experiment when compare to the others of which the plants treated with *Pseudomonas fluorescens* and those treated with *P. putida* lived up to 35 days while the control (plant not rhizo-injected with any test organism) could not live beyond 25 days. This indicates an increase in life span by 40 percent in the treatment with either of *Pseudomonas putida* and *Pseudomonas fluorescens* and 80 percent in treatment with a combination of both *Pseudomonas putida* and *Pseudomonas fluorescens* when compared to the control. This is in agreement with the ability of the PGPR strain of *Pseudomonas spp.* to help in the alleviation of various type of stress as reported by Ruzzi and Aroca, 2015. One of the mechanisms that could have been responsible for this delay in the death is the ability of the *Pseudomonas spp.* to induce stress tolerance in the plant by the releases a wide range of secondary metabolites and volatile organic compounds (VOCs) produced by *Pseudomonas spp.* can improve stress-tolerance and stimulate growth in plants. It can also help to activate stress tolerance gene in the plant (Bailly and Weiskopp, 2012).

Plants in the humus soil under different treatment with the test organisms showed differences in the final readings taken for parameters such as height, leaf area, number of leaves, stem girth, length of root and root dry weight.

At the end of the experiment, plant treated with *Pseudomonas spp.* were of almost equal height as the untreated ones except for about 3cm increase in the rhizo-inoculated plants. However other plant parameters showed significant difference.

Result on the leaf area shows difference in the response of *Amaranthus hybridus* same as *Amaranthus viridis* to the different forms of treatment with *Pseudomonas spp.* All the plant treated with pseudomonas shows increase in leaf area, however the plant treated with the combination of both *Pseudomonas putida* and *Pseudomonas fluorescens* has the highest surface area. When the various treatment was compared with the control, the plant with the *Pseudomonas putida* and *Pseudomonas fluorescens* combination has an increase of about 29 percent in leaf area while those treated with *Pseudomonas fluorescens* made an increase of about 14 percent, while the minimal increase was about 7 percent in plants treated with *Pseudomonas putida*. This is probably an indication that the *Pseudomonas spp.* had mediated the leaf formation.

When the roots of the plants were collected and checked, it was found that the length of all treated plants were longer than those of the control plants.

Result on the root parameter after 65 days of the experiment shows significant increase in the primary root length and also increase in the weight of the dry weight of the plants treated with the test organisms. 100 percent increase in the root length was in the plant treated with a combination of *Pseudomonas putida* and *Pseudomonas fluorescens* (this the mass is double that of the control), followed by plant treated with *Pseudomonas putida* which shows an increase by about 92 percent and the plants treated with *Pseudomonas fluorescens* which shows an increase by 87 percent when compared with the control. A corresponding increase in the dry weight of the root was also presented by the same table with the highest weight measured in the plants treated with *Pseudomonas fluorescens* which shows an increase of

about 569 percent dry weight, followed by the plants treated with a combination of both *Pseudomonas putida* and *Pseudomonas fluorescens* with a corresponding increase of about 549 percent and lastly by the plants treated with *Pseudomonas putida* which shows an increase of about 508 percent dry weight. This increase in length and weight of the root can be related to the report given by Vacheron *et al.*, 2013. which support enhancement to root parameter as one of the ways by which PGPRs enhances the growth of plants. The mechanism behind this could have been that the *Pseudomonas spp.* have stimulated. They can improve root development and growth through the production of phytohormones or enzymatic activities.

The flowering parameter at the last day also shows an increase in the length of flowers in the plant treated with the test organism. Plant treated with the combination of both test organism (*Pseudomonas putida* and *Pseudomonas fluorescens*) had the longest inflorescence length of 11.9cm, followed by treatment with *Pseudomonas fluorescens* with inflorescence length of 11.2cm, treatment with *Pseudomonas putida* with corresponding inflorescence length of 10.6cm while the control had inflorescence length of 8.0cm. when the various treatment was compared with the control, the treatment with the combination of *Pseudomonas putida* and *Pseudomonas fluorescens* showed an increase of about 49 percent, 40 percent increase in plant treated with *Pseudomonas fluorescens* while *Pseudomonas putida* had about 33 percent increase. However, irrespective of the inflorescence length at the end of the experiment, the onset of inflorescence was first observed in the plants treated with the combination of *Pseudomonas putida* and *Pseudomonas fluorescens* on the 47<sup>th</sup> day, followed by the control on the 48<sup>th</sup> day. Unexpectedly, plants treated with either of *Pseudomonas fluorescens* or *Pseudomonas putida* produced inflorescence last which was on the 50<sup>th</sup> day. The increase in the length of the inflorescence was in agreement with the report by Esitken (2006), where it

was stated that biopriming increases both the floral and foliar yield in plant because of the hormone.

The overall enhancement of the growth parameters would also confirm the ability of *Pseudomonas spp.* to enhance the growth of *Amaranthus hybridus* and *amaranththus viridis*

### **5.3 Impact on germination**

On the field, the red soil could not support the growth of *Amaranthus hybridus* and *Amaranthus viridis*, it became necessary to check the effect of red soil filtrate on the germination of the plant. It was found out that the behavior of germinant in the red soil filtrate was just like every other treatment. However, because of the difference in the soaking period of the seeds in the various test organism suspension solution before planting, it was observed that the seeds soaked in 24 hours in the test organism before planting had a better percentage germination when compared to the seed soaked for 3 hours before planting irrespective of the treatment with test organism. The greatest difference in germination percentage for the soaking period was observed in the seeds soaked in water and planted in petri dishes moistened with water. The seeds soaked for 24 hours had about 91.8 percent germination while seeds soaked for 3 hours had about 51.9 percent germination. Minimum difference was observed in the seed bio-primed in the suspension of *Pseudomonas putida* and sown in petri dished moistened with humus soil filtrate, where the seeds soaked for 3 hours and 24 hours had about 83 and 86 percent germination respectively.

In the seeds soaked for 24 hours and sown in the petri dished moistened with red soil filtrate seeds bio-primed with *Pseudomonas fluorescens* had the highest germination percentage followed by seed bioprimed with *Pseudomonas putida*, a combination of *Pseudomonas fluorescens* and *Pseudomonas putida*. The control had the minimum germination percentage germination. Higher germination was also presented in the seed bio-primed and sown in humus soil filtrate than the unprimed seeds sown in the humus soil filtrate, with seeds treated

with *Pseudomonas putida* had the highest germination percentage. However, in the petri dishes moistened with distilled water, seeds treated with *Pseudomonas putida* had the lowest germination percentage when compared to other bio-primed seeds as well as the control. In the 3 hours soaking period, unprimed treatment had the lowest germination percentage in all the petri dishes, with the lowest in the red soil filtrate. In treatment with distilled water biopriming with the combination of *Pseudomonas fluorescens* and *Pseudomonas putida* had the highest germination percentage while treatment with *Pseudomonas putida* and *Pseudomonas fluorescens* had the same germination percentage. In the treatment with humus soil, biopriming with *Pseudomonas putida* had the highest germination percentage, followed by biopriming with *Pseudomonas fluorescens* and lastly biopriming with the combination of *Pseudomonas fluorescens* and *Pseudomonas putida*. In the treatment with red soil filtrate biopriming with *Pseudomonas putida* showed the highest germination percentage, followed by biopriming with the combination of *Pseudomonas fluorescens* and *Pseudomonas putida* and lastly biopriming with *Pseudomonas fluorescens*. The percentage generally reflected that percentage germination was increased when the soaking period was 24 hours before sowing and there was a relative reduction in the treatment with 3 hours soaking period. This is in conformity with the report given by Kochoni, *et al*, (2013), which has it that soaking the seeds for a period of 12 hours enhanced the germination rate of *Momordica charantia* L. the mechanism involve has been said to be related to activation and produces enzymes like amylase and lipase which activate storage materials in seeds which is responsible for the early germination and further growth of the seedling.

Result from the leaf length of germinant in the petri dishes moistened with water showed no differences in the length of leaf for all treatment except for the treatment with *Pseudomonas fluorescens* which has a length of 3.1mm while other has 3.0mm regardless of the time of soaking. In the petri dishes with humus filtrate the highest length of leaf was record in the

germinant treated with *Pseudomonas putida* and *Pseudomonas fluorescens* with the soaking period of 24 hours and 3 hours respectively before germination (3.3mm), while the minimum height was recorded in the control with 3 hours soaking period before germination (3.0mm). In the red soil filtrate treatment, the highest leaf length was 3.3 and was recorded in the germinant treated with *Pseudomonas putida* and a combination of *Pseudomonas putida* - *Pseudomonas fluorescens* treatment in the soaking period of 24 hours while the minimum was 2.8mm and was recorded in the unprimed germinant with the soaking period of 3 hours. It was observed that the best of the leaf length was recorded in the germinant bioprimered in the test organism irrespective of the soaking period in red soil and humus soil filtrate. This is an indication that the test *Pseudomonas spp.*, have improved the growth of the germinant by formation of leaves as reported by Noumavo *et al.*, (2013). This increase in leaf length may be due to the production and metabolism of auxin, hormones responsible of the cellular elongation or cytokinin, hormones that stimulate the cellular division triggered by PGPR treatment.

The height of the shoot of the germinant treated with the test organism had an increase by 40, 15 and 13.5 in the germinant bioprimered with *Pseudomonas fluorescens*, *Pseudomonas putida* and a combination of *Pseudomonas fluorescens* and *Pseudomonas putida* respectively in the petri dish moistened with water and presoaked for 24 hours. In the 3 hours soaking component, the control had a shoot length higher than the germinant bioprimered in the combination of *Pseudomonas putida* and *Pseudomonas fluorescens*, but had lesser height length than those bioprimered in either *Pseudomonas putida* or *Pseudomonas fluorescens*. In the petri dishes moistened with humus soil filtrate irrespective of the treatment of the test organism and the time of soaking in the test organism, the control had longer shoot than the other treatments. In the petri dishes moistened with red soil filtrate, irrespective of the soaking period before sowing, only the germinant treated with either

*Pseudomonas putida* or *Pseudomonas fluorescens* had higher length than the control by 13 and 4 percent in those with the soaking period of 24 and 3 hours respectively. The germinant treated with the *Pseudomonas fluorescens-Pseudomonas putida* combination had less height than the control. The increase in shoot length reported is in accordance with the report by Widawati and Suliasih, 2018. The increase could have been because of production of auxin by the *Pseudomonas spp.*, which is responsible for the promotion of growth by elongation.

At the end of the germination experiment, some of the germinant showed lodging by way of bending. Although, many germinant were shown to lodge during the course of the experiment. The highest germination percentage was shown in the germinant treated with the combination of both *Pseudomonas putida-Pseudomonas fluorescens* and sown in the petri dishes moistened with red soil filtrate and bioprimering period of 24 hours with 93 percent lodging, while the lowest lodging was found in the petri dishes moistened with water and without water with soaking period of 3 hours before sowing. The germinant that showed high percentage lodging (80.8-93.4) were found in the petri dishes bioprimered and had seed soaked for 24 hours before sowing. This could be an indication that lodging was as a result of the plants weight growing beyond what the tender stem can carry. It could also be as a result of promoted growth by a factor of bioprimering and 24hours soaking period. This could however, be related to the ability of the *pseudomonas spp.* to increase the biomass of plants as reported by Xia (2019).

When the effect of the test organism treatment was compared, it was observed that the combination of two species of the test organisms and the use of only *pseudomonas fluorescens* had the best impact in growth promotion against the use of only *Pseudomonas putida*. Qualitative assessment of phytochemicals showed presence of saponins, phenolic, tannins, terpenoids, alkaloids, and flavonoids in both species notwithstanding inoculation. The presence of glycosides in plants inoculated with *P. pseudomonas* was reported.

Glycosides were absent in the control plants. Significant reduction in total phenols upon inoculation of plants with *P. putida* was also reported. Following the failure of FRS-exposed plants to subsist, in-vitro growth studies were conducted to compare seed growth response. Results showed that seeds sown in FRS-filtrates grew as better as those in HMS-filtrates. Germination percentage in the *Pseudomonas*-exposed seeds was better when seeds were primed for 24h than ofr 3 h in both plant species.

#### **5.4 Conclusion**

In this present study, it has been established that the use of *Pseudomonas spp.* has the ability to promote the growth parameters of *Amaranthus hybridus* and *Amaranthus viridis* when humus soil is used. The test organisms have also shown ability to increase stress tolerance in the test plant, however, the various species have failed to promote the growth of *Amaranthus hybridus* and *Amaranthus viridis* up to such a level which it could be presentable for consumption when planted in ferruginous soils. This is an indication that other method should be looked into in other to be able to enhance the cultivation of *Amaranthus hybridus* and *Amaranthus viridis* in stress condition such as in ferruginous soil. It is also important that research should be channeled to look into the reason why the *Pseudomonas spp.* could not promote the growth of *Amaranthus spp.* in ferruginous soil and how the barrier could be by passed.

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