ENHANCING SOIL AVAILABLE PHOSPHORUS VIA MICROBIAL INOCULATION IN AN ULTISOL

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DEPARTMENT OF SOIL SCIENCE AND LAND MANAGEMENT

FACULTY OF AGRICULTURE

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BENIN CITY

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A PROJECT REPORT SUBMITTED TO THE

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CERTIFICATION

This is to certify that this work **"Enhancing Soil Available Phosphorus Via Microbial Inoculation In An Ultisol"** was carried out by **Miss Ebosetale Judith EHIAGWINA (AGR1500296)** of the Department of Soil Science and Land management, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

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DEDICATION

This work is dedicated to God Almighty for granting me success and to my amazing parents, Mr& Mrs Ehiagwina who ensured I had the best of everything.

ACKNOWLEDGEMENT

My sincere gratitude goes to God Almighty. To my parents, Mr & Mrs Ehiagwina for their prayers, support and encouragement. Thank you. Having wonderful siblings who love you is simply amazing, I am indeed grateful to my siblings. They are the absolute best.

A profound appreciation goes to my project supervisor, Mr I. Ogbemudia for his careful scrutiny of this work. He always had time to look at my write up and make corrections, your type is indeed rare. To the present Head of Department of Soil Science and Land Management, Dr (Mrs) A.O Bakare for her motherly care. To Prof J.S Ogeh for your wonderful counsels. To my course adviser, Miss F.E Okunsebor, for helping me become a better student. Thank you.

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Life is indeed boring without having friends. A deep appreciation goes to my friends. Thank you, Valentina for being so understanding and always listening to me. I love you. Thank you Uyo for always checking up on me and your words of encouragement, you have a special place in my heart. A big appreciation goes to my project colleagues Wisdom, John- Paul and Mercy. Everything seemed so easy despite those months of intense pressures. It was a seamless transmission and I'm most happy for that.

How can I forget my very own Very Rev Fr.Dr. Andrew Ovienloba-Ihonda for his spiritual support and his numerous pep talk. Thank you Padre.

May the good Lord bless and reward you all. Amen.

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ABSTRACT

The research was carried out at the Faculty of Agriculture, University of Benin to investigate the availability of soil phosphorus resulting from microbial inoculation in an Ultisol with maize as the test crop. The experiment was laid out in a Randomized Complete Block Design (RCBD) in three replications comprising; T_1 (control), T_2 ($NPK_{15:15:15}$) and T_3 ($NPK_{15:15:15}$ + *Bacillus sp).,* to give a total of nine experimental plots measuring 2.5m x2.5m used for the investigation. Results from the work showed a significant effect (P=0.05) of NPK + *Bacillus sp* on the grain yield (8.7g/18.75m^2) of maize than that of the uninoculated treatments (7.7g/18.75m^2- 6.6g/18.72m^2); while no significant differences were observed in the plant parameters measured until week8 where the leaf area (509.0 cm^3) of the inoculated plants (T_3) was significantly better than the values observed for T_1 (350.7 cm) and T_2 (420.3cm) respectively. Available P in soil also increased on the inoculated (20.01 $mgkg^{-1}$) treatment than those not inoculated (8.65 – 19.42 $mgkg^{-1}$). Although the microbial population (9 x 10^3 cfu) in the control (T_1) was relatively higher than the population in T_2 (4 x 10^3) and T_3 (7 x 10^3), bacillus sp as a promising P – solubilizer was dominant (50 percent) in the inoculated soils (T_3) than the uninoculated.

CHAPTER ONE

I. INTRODUCTION

Phosphorus is a fundamental mineral nutrient vital for Agricultural, Crop development and important in physiological processes (Krishnaraj and Dahale, 2014). Phosphorus is known to promote root development, rapid plant maturity, seed production, resistance to diseases and as well as improve water use. (Murrell and Munson, 1999). Phosphorus represents about less than 0.20% of total plant dry weight and about 0.05% of soil content, which only a small amount is bioavailable for plants (Alori, 2017). Nevertheless, the amount of readily available Phosphorus is very low compared with the total amount required by plants.

Most acid soils particularly Ultisols have the problems of low organic matter, low availability of macro-element and low soil Phosphorus. (Eswaran *et al.,* 1997). Acid soils mainly ultisols are very common and abundant in distribution. These soils have high Phosphorus-fixing capacity hence, it requires intensive Phosphorus fertilization rates for obtaining economical yields and have more than half of their total Phosphorus as Organic Phosphorus (Razaq *et al.,* 2017).

Due to the fact that millions of tons of Agrochemicals including Phosphorus fertilizers are used indiscriminately to achieve optimum crop yields, thereby disrupting the composition of rhizosphere organisms, soil matrix, human health and lastly food chain. It became increasingly important to find low-cost alternative like the utilization of renewable resources, this led to the discovery of plant growth promoting Microorganisms (Microbial Inoculants). There is only a small amount of soil phosphorus available for plant whereas the need to introduce microbial inoculants which have both beneficial and endophytic abilities to promote and induce plant health became very important.

Agriculture is challenged by increases in global demand for food, alongside multiple pressures from the environment. All these need to be managed in an environmentally-friendly processes. There is need to provide answers to address nutrient limitation of Phosphorus hence the need for this study.

The general objective of this study is to determine the influence of a known Phosphorus Solubilizing Bacteria on the availability of soil phosphorus in an ultisol with maize as the test crop.

1.1 SPECIFIC OBJECTIVES

The specific objective of this study were to determine the:

1) physical and chemical properties of the soil.
2) influence of *bacillus sp* on Phosphorus mineralization in an utltisol.
3) effect of the solubilizing organism on the yield of maize.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil Phosphorus and its nature

Phosphorus is one of the most abundant metallic elements found in the earth. (Gyaneshwar *et al.,* 2002). Phosphorus (P) is essential to all forms of life on this planet. It is an essential nutrient necessary for growth and development of plants on which our food supply depends solely. (Azziz *et al.,* 2012., Tak *et al*., 2012). Having about 0.5% of a plant's dry weight, Phosphorus is involved in an array of processes in plants such as photosynthesis, respiration and energy generation. (Vance *et al.,* 2003). It as well helps in cell division, resistance to plant diseases (Khan *et al.,* 2014), enzymatic activation and inactivation and carbohydrate metabolism. (Razaq *et al.,* 2017). Despite its importance in plants growth and metabolism, Phosphorus is the least available macro-nutrient and the most frequently deficient nutrient in most agricultural soils due to the low availability of phosphorus and its poor recovery from the application of fertilizers (Kang *et al.,* 2011). Its limitation negatively impacts crop yield and quality. Phosphorus deficiency is addressed through application of culturally available P-fertilizers. Also, global Phosphorus reserves have a higher depletion and reports estimates that there will be no Phosphorus reserved by the year 2050 (Cordell *et al.,* 2011).

For sustainable crop production to be achieved there has to be maintenance of high crop yield without affecting ecosystems to meet the current yield. (Tilman *et al.,*2002).

Plant roots can only acquire Phosphorus from the soil when it is dissolved in soil water. Phosphorus is a reactive element which does not exist as elemental form in the soil. Insoluble organic Phosphorus and insoluble inorganic Phosphorus are the only forms in which Phosphorus

exists in the soil. (Walpola and Yoon, 2012). Most transformations of inorganic forms in the soil are controlled by the action of precipitation, dissolution and sorption. Only very low concentration of Phosphorus is in the soil water, Phosphorus is continually replenished from soil mineral and as well as organic matter to replace the Phosphorus which was taken up by the plant. (Khan *et al.,* 2009), (Sharma *et al.,* 2013).

The Phosphorus level in the soil is usually put at 0.05% (Sharma *et al.,* 2013). Tested values of soils are much higher but a great part of it is about 95% which is present in the form of insoluble phosphate (Pradhan and Sukla, 2005). Plant cells might take up several P forms, but a greater percentage is absorbed in phosphate anions forms HPO_4^{2-} or $H_2PO_4^-$ depending on the soil pH (Satyaprakash *et al.,*2017), (Kumar *et al.,* 2018).

2.2 Essential Roles of Phosphorus in Plants

Phosphorus is vital component of nucleic acids **DNA** and **RNA**. **DNA** which is the genetic "memory unit" of all living things is the component of **RNA**, the compound that the DNA genetic code uses to build proteins and various compounds essential for plant structure, seed yield and genetic transfer. The structures of both DNA and RNA are linked together by phosphorus bonds. (Elser *et al.,* 2010) and (Suzuki *et al.,* 2010)

Phosphorus is a component of ATP, "the energy unit" of plants. ATP forms during photosynthesis, has Phosphorus in its structure and processes of seed growth through the formation of maturity. (Zhu and Smith, 2001) (Nadeem *et al.,* 2012).

Phosphorus is essential for the vigor and overall health of plants. Some specific growth and developmental roles are:

- **Seed Germination**

  Phosphorus is an important factor for seed germination, improved seedling. P is only made available to plants during germination and it also helps in the support of early seed growth. The plant requirement of P is met from growing media through roots. (Zhu and Smith, 2001).

- **Flower and Seed formation**

  Phosphorus is an active participant in improving the reproductive growth of plants which including flower and seed formation. P contributes in the production of anthocyanins in the stalks of flowers (Chen *et al.,*2013). There are large quantities of P which are found in fruits and seeds where it is important for seed formation and the overall development. Where there is no adequate amount of P seed size, seed number and lastly viablilty is drastically reduced. (Soares *et al.,*2016).

- **Increasing Root and Shoot Strength**

  The growth of plants right from cellular to whole plant level is an important function of Phosphorus. The growth parameters include plant height, leaf area and number and shoot dry biomass. (Assuero *et al.,*2004). P also plays an important role in cell division and enlargement and as well as timely appearance of tillers in crop plants.

2.3 Soil Phosphorous Availability/Deficiency

Soil phosphate can be rendered unavailable for plant roots and P is the most immobile of the entire plant nutrients (Satyaprakash *et al.,*2017). Soils with strong phosphate fixation require higher fertilizer phosphate application to reduce the effects of fixation. If the available quantity

of soil phosphate is normal in terms of its range, the rate of phosphate application required should correspond to the amount being removed by the crop (Walpola *et al.,*2012).

For plants that have high absorption rate, it is necessary that the phosphates in solution must be replenished several times per day by the mobilization of phosphate. The absorption rate is higher in soils with a higher buffer capacity (Kumar *et al.,*2018). Soluble Phosphorus either from natural weathering or from fertilizer reacts with iron, clay and aluminum compounds which are present in the soil and is readily converted to less available forms by the Phosphorus fixation process (Sharma *et al.,* 2013). As a result of these various processes, Phosphorus moves very slowly in most soils due to the loss of P through leaching (Mahidi *et al.,* 2011). The fixed, residual phosphorus remains in the rooting zone and it will be slowly available to succeeding crops (Chen *et al.,* 2006). The significant ways in which soils are lost includes; Soil erosion and Crop removal.

Inherent soil properties and climate affect the growth of crops and their responses to applied P fertilizer.

The first indication of Phosphorus shortage is often a stunted plant, which is difficult to diagnose. Distorted leaves shapes with severe deficiency, dead areas may develop on leaves, fruits and stems. (Zambrosi *et al.,* 2014). Older leaves are affected before younger ones because of Phosphorus distribution within the plant. Plants such as maize may display a purple color on the lower leaves when Phosphorus is low (Hinsinger, 2001).

Other changes including reduction of primary root length, root biomass, root hair density and length, formation of cluster roots, alongside greater root penetration capacity (Jungk, 2001) are

as a result of Phosphorus limitation. In general, changes induced by P deficiency causes changes in the physiological, biochemical and metabolic activities. (Hinsinger, 2001)

2.4 Microbial Inoculation

Microbial inoculants are natural based products which are widely used to control pests and improve the quality of the soil and crop. These inoculants are environmentally friendly and deliver nutrients to the plants in a very efficient manner. These are agricultural amendment that utilize rhizospheric or endophytic microbes to promote the health of plants (Deubel *et al.,*2000). Many of the microbes which are inoculants form symbiotic relationships (Sullivan, 2001) and the benefits of inoculation in agriculture extends beyond biofertilizers. Microorganisms play a vital role in promoting microorganisms (PGPMs). PGPMs are generally attributed to three mechanisms which are:

1. PGPMs acting as biofertilizers such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria. (Kennedy and Islam, 2001).
2. Phtostimulators such as *Azospirllum* which directly promotes the growth of plants by the production of plant hormones. (Glick *et al.,* 2007).
3. It also acts as biological control agents such as *Pseudomonas* for the protection against plant pathogenic organisms. (Mohiddin *et al.,*2010) (Dawar *et al.,* 2010).

2.5 Phosphorus Solubilizing Microorganisms (PSM)

PSM dates back to 1903 (Khan *et al.,* 2009). Microbial species which play key roles in P solubilization includes bacteria, fungi, actinomycetes and even algae. Bacteria are predominantly present and it has been proved to be effective in phosphorus solubilization than fungi (Wani *et al.,* 2005) hence concentration is placed on it in this review. Phosphorus Solubilizing Bacteria

constitutes about 1-50% in the microbial population in the soil making it the most abundant (Chen *et al.,* 2006). Furthermore, the organisms possessing a phosphate-solubilizing ability can also convert the insoluble phosphate compounds into soluble forms (Kang *et al.,* 2002).

Many species of soil fungi and bacteria are able to solubilize phosphorus *in vitro* and a good number of them can release Phosphorus in plants (Zhu *et al*; 2011). PSM leads to an increase in the bioavailability of soil insoluble phosphorus for plants utilization (Zhu *et al;* 2011). They are capable of solubilizing insoluble mineral phosphorus and mineralize organic phosphorus (Sharma *et al.,* 2013). The salt-tolerant microorganisms that also exhibit the ability to solubilize insoluble phosphorus facilitate the gradual development of saline-alkaline-soil based Agriculture (Zhu *et al.,* 2011). The inoculation of soil with phosphate mineralizing and solubilizing microorganisms is a strategy for the improvement of plant absorption of phosphorus and thereby limiting the usage of chemical fertilizers (Alori *et al.,* 2012).

Phosphorus mineralizing bacteria includes various strains of *Azotobacter*, *Erwinia* (Chakraborty *et al*., 2009) *Rhodococcus, Serratia, Bradyrhizobium, Salmonella, Sinomonas Thiobacillus* (Postma *et al.,*2010; David *et al.,* 2014).

Phosphate Solubilizing Microorganisms have considerable synergistic effect on the growth and overall development (Tallapragada and Gudimi., 2011). In as much as some solubilizing Phosphorus microorganisms also solubilize Phosphorus, they also demonstrate potential as some agent of bio-control agents against some pathogenic plants.

PSM manages these pathogens by producing a sort of antifungal compounds (such as phenolics and flavonoids) all of which enhance inhibition of the growth of plant pathogens.

PSM technology also improves the fertility use of saline soil without necessary causing any harm or any environmental implication that accompanies the use of continuous application of synthetic fertilizers (Zhu *et al.,* 2012).

The PSM *Burkholderia cepacia* promotes the growth of maize plants in the presence of Sodium Chloride concentrations of up to 5% (Zhao *et al.,* 2014). In one set of experiment for bacterial solubilization, increases in NaCl concentration till about 0.8m resulted in the percentage increment in the percentage of Phosphorus released but it was declined. On the other hand, with increases in NaCl concentration the amount of Phosphorus released among phosphate solubilizing fungi which increased throughout the incubation periods (Srinvisan *et al.,* 2012).

The PSB solubilize the fixed soil P and applied phosphates leading to a higher crop yield. Direct application of phosphate rock is often ineffective in the short time period of most annual crops. Microorganisms that produce acids are able to enhance the solubulization of phosphatic rock. The PSB strains exhibit inorganic P-solubilizing abilities ranging from 25-42µg PmL^{-1} and organic P mineralizing abilities between 8-18µg PmL^{-1} (Tao *et al.,* 2008). The PSB in combination with single super phosphate reduces the P dose by 25 and 50% respectively (Sundara *et al.,* 2002).

The symbiotic relationship between PSM and plant is synergistic in nature as there is provision for bacteria to supply soluble phosphate. Plant supply root borne carbon compounds (mainly sugars) which can be metabolized for the bacterial growth. The PSM along with other beneficial rhizospheric microflora enhances crop production. Simultaneous application of *Rhizobium* with PSM has been shown to enhance plant growth more than their sole inoculation in certain situations when the soil is P deficient. Synergistic relationship on plant growth has been observed by the co-inoculation with N2 fixers such as *Azotobacter.* (Zaidi *et al.,* 2003).

2.5.1 Factors Influencing Microbial Phosphate Solubilization

The capability of PSM to successfully transform insoluble organic and inorganic phosphorus is associated with the nutritional value, richness of the soil, the growth and physiological status of the organism. PSM from soils of extreme environments which includes cases of soils with high level of nutrient deficiencies, saline-alkaline soils and soils with extremely low temperature have the tendency to solubilize more phosphate than PSM from soils with more moderate conditions (Zhu *et al.,* 2011). There have been conflicting reports on the influence of temperature for maximum microbial phosphorus as reported by (Kang *et al.,* 2002) and as well as (Varsha, 2002). Furthermore, reports collected from Fasim *et al 2002)* and (Nautiyal *et al.,*2000) emphasized on the extreme temperature of 45^0 C of P solubilization in desert soil.

Among other factors influencing microbial phosphate solubilization includes;

- Land use systems
- Plant types
- Climatic zone types
- The extent of vegetation
- Lastly, the soil's physiochemical properties which includes organic matter and soil pH (Seshachala and Tallapragada., 2012).

Solubilization of Phosphorus is enhanced quickly in warm humid climates and it is usually slow in cool dry climates. Rapid phosphorus solubilization is more readily permitted compared to saturated wet soils. Zhang *et al.,* (2014) reported that additions of small amount of inorganic phosphorus to the rhizosphere could drive phytic acid mineralization by bacteria improves phosphorus- plant nutrition. Phosphorus Solubilizing Bacteria population richness and diversity

according to Azziz *et al*., (2012) were more abundant and diverse following crop rotation. Soils that are rich in organic matter will favor microbial growth which consequently favors Phosphorus solubilization.

pH of soils between 6 and 7.5 are best for P-availability due to the fact that at pH values of 5.5 and between 8.5 limits P from being fixed by aluminum, calcium and even iron, hence, not being available for plant utilization. (Zhao *et al.,* 2014) made a negative correlation between the amount of phosphate solubilized by B. *cerpacia* SCAUK0330 as well as the pH decline in the process. Research has also shown that microbial phosphate solubilization depends largely on the kinds of metabolite produced and its rate of release. (Zhu *et al.,* 2011).

2.5.2 Mechanism of Inorganic Phosphate Solubilization by Phosphate Solubilizing Microorganisms

As observed in many experiments, the principal mechanism is the production of dissolving mineral compounds such as hydroxyl ions, CO_2 siderophones, organic acids. (Rodriguez and Fraga, 1999; Sharma *et al.,*2013). Organic acids produced together with their carboxyl and hydroxyl ions reduce the basicity and alkalinity to release P. (Seshachala and Tallapragada, 2012). Other mechanisms of mineral phosphate solubilization by microorganisms are the production of inorganic acids (such as nitric acid) and the production of chelating substances in the release of Phosphorus in soil is less than that of the organic acids. (Kim *et al.* 1997) therefore reiterates that organic acid production in P-Solubilization by PSM is not the only reason for the increment in the Phosphorus concentration into culture medium. Consequently, Mycorrhizal fungi effectively extend plant roots, aiding crop phosphorus nutrition by increasing the volume of soil from which phosphate may be absorbed (Browne *et al.,* 2009).

Another mechanism of microbial phosphate solubilization reported is the liberation of enzymolysis, the mechanism of P solubilization by PSM in a medium composed of lecithin where the increase in acidity is caused by enzymes that act on lecithin (Zhu *et al.,* 2011).

2.5.3 Mechanisms of Organic Phosphorus Mineralization

The major source of organic Phosphorus in the soil is the organic matter. The values of organic phosphorus in soil can be as high as 30-50% of the total P and soil organic P is largely in the form of soil phytate (Rodriguez and Fraga, 1999). In addition, large quantities of xenobiotic pesticides, detergents additives are regularly released into the environment containing organic P as its composition.

(Peix *et al.,* 2001) stated that most organic compounds have molecular-weight materials that are generally resistant to chemical hydrolysis and must therefore be bio-converted to either soluble ionic phosphate (Pi, HPO_4^{2-}, $H_2PO_4^-$,) or low molecular-weight organic phosphate, to be assimilated by the cell.

Phosphorus mineralization refers to the solubilization of organic phosphorus and the degradation of the remaining part of the molecule. (Halvorson *et al.,* 1990) proposed one theory about the solubilization of organic phosphorus which is the Sink Theory. It refers to the continuous removal of P results in the removal of Calcium-Phosphorus compounds. Different groups of enzymes are involved in the biological process in Phosphorus Cycling. The first group of enzymes are dephosphorylates. They are non-specific acid phosphates (NSAPs) enzymes. The most studied among these released NSAPs are the Phosphatases (Nannipieri *et al.,* 2011).

Lastly, another enzyme produced by PSM is the enzyme responsible for the release of phosphorus from organic materials in soil that are stored in the form of phytase. Phosphorus is

released in a form of phytase degradation by phytase that is made available to plants. Plants generally cannot acquire phosphorus directly from phytase, however due to the presence of PSM within the rhizosphere, there may be a compensation for a plant's inability to acquire phosphorus directly from phytase (Richardson and Simpson, 2011).

2.6 Phosphorus Solubilizing Bacteria (PSB)

Among the phosphorus solubilizing microorganism in the soil, PSB constitutes about 50%. High proportion of PSB is concentrated in the rhizosphere. PSB are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds (Chen *et al.,* 2006). P-Solubilization ability of rhizosphere microbes is considered to be one of the most vital traits associated with plant phosphate nutrition (Malboobi *et al.,*2009). The PSB are ubiquitous with variations in forms and populations in different soil. The population of PSB depends on their different soil properties which are the physical and chemical properties, organic matter and P content and their cultural activities. A greater population of PSB is found in Agricultural and rangeland soils. The PSB count ranged from 0-107cells with 3.98% population of PSB among total bacteria (Fallah, 2006). Species of plant with plant developmental stage and soil type have thus been indicated as major factors determining the composition of biofertilizers. PSB have attracted the attention of Agriculturists as soil inoculums to improve the plant growth and yield (Bass *et al.,*2016).

2.6.1 Mechanisms of Phosphorus Solubilizing Bacteria

Some bacterial species have potentials for mineralization and solubilization in organic and inorganic phosphorus respectively. Phosphorus solubilizing activity is determined by the capability of microbes to release metabolites which include organic acids through the utilization

of their carboxyl and hydroxyl groups chelate the cation bond to phosphate, the carboxyl is converted to soluble forms. A wide range of microbial P solubilization mechanisms exist in nature and a considerable amount of the global cycling of insoluble organic and inorganic soil phosphate is generally considered to be bacteria. A large proportion of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates is responsible for phosphorus solubilization mainly through the action of chelation-mediated organisms (Whitelaw, 2000).

Solubilization of Inorganic P is enhanced by the action of inorganic and organic acids secreted by PSB in which hydroxyl and carboxyl groups chelate cations and decrease the pH in basic soils. (Stevenson, 2005). The PSB dissolves the soil phosphorus via production of low molecular weight organic acid mainly gluconic and keto gluconic acids (Deubel *et al.,* 2000). Inorganic acids for example hydrochloric acid can also solubilize phosphate but are relatively less effective compared to organic acids at the same rate of pH. In some cases, phosphate solubilization is induced by phosphate starvation (Gyaneshwar *et al.,* 2002).

2.6.2 Solubilzation of Calcium- bound P

Apatites and metabolites of phosphorus-based fertilizers are fixed in the form of calcium phosphates under alkaline conditions. A large number of the calcium phosphates including rock phosphates ores are insoluble in soil with respect to the release of inorganic P. Combined effect of pH decrease of soil pH and organic acids production is the reason for Phosphate Solubilization. (Fankem *et al.,* 2006). Microorganisms through the secretion of different types of organic acids produced carboxylic acid (Dubel *et al.,* 2000). And rhizospheric pH lowering mechanisms dissociate the bound forms of phosphate like $Ca_3(PO_4)^2$. Moreover, buffering capacity of the medium reduce the effectiveness of PSB in releasing P from tricalcium phosphates. (Stephen and

Jisha., 2009). Acidification of the microbial cell surroundings releases P from apatite by the substitution of proton and also the exclusion of H^{+}. (Vilegas and Fortin., 2002).

Complexing of cations is a necessary tool in P solubilization and it is controlled by nutritional, physiological and growth culture of the microbe.

2.6.3 Solubilization of Al/Fe bound P

Fe and Al solubilization occurs via proton release by PSB by decreasing the negative charge of absorbing surfaces to aid the process of sorption of negatively charged P ions. Proton release can also decrease the P sorption upon acidification which increases $H_2PO_4^-$ in relation to $HP0_4^{2-}$ having higher affinity to reactive soil surfaces. Carboxylic acids often solubilize through direct dissolution of mineral phosphate as a result of anion exchange of $P0_4^{3-}$ by acid anion, or chelation of both Fe and Al ions associated with phosphates. Ability of organic acids to chelate metal cations is greatly influenced by its molecular structure, particularly by the number of carboxyl and hydroxyl groups. Type and position of the ligand in addition to acid strength determine its effectiveness in the solubilization process. Phosphorus desorption potential of various carboxylic anions lowers with decrease in stability in stability constants of Fe or Al organic acid complexes. (Ryan *et al.,* 2001).

2.6.4 Mineralization of Organic Phosphorus

Mineralization of soil organic P plays a vital role in the cycling of Phosphorus of a farming system. Organic P may constitute about 4-90% of the total soil P. Usually half of the microorganisms in the soil possess P mineralization potential. Acid and alkaline phosphatases utilize organic phosphatases as a substrate to convert it into inorganic form. The principal mechanism for mineralization of soil organic P is the production siderophones and acid

phosphatase by plant microbes from organic residues. The biggest portion of extracellular soil phosphatase is gotten from the population of microbes. (Dodor and Tabatabai., 2003).

2.8.5 Interaction of PSB with other Microorganisms

Symbiotic relationship between PSM and plant is synergistic in nature as there is provision for bacteria to supply soluble phosphate. Plant supply root borne carbon compounds (mainly sugars) which can be metabolized for the bacterial growth. The PSM along with other beneficial rhizospheric microflora enhances crop production.

Simultaneous application of rhizobium with PSM has been shown to enhance plant growth more than their sole inoculation in certain situations when the soil is P deficient. Synergistic relationship on plant growth has been observed by the co-inoculation with N2 fixers such as *Azotobacter.* (Zaidi *et al.,* 2003).

2.6.5 Effect of PSM on Crop Production

Use of PSMs can increase crop yields up to about 70%. Combined inoculation of *arbuscular mycorrhiza* and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (Cabello *et al.,* 2005). Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB (Zaidi *et al*., 2003).

Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation. *Pseudomonas sp* enhanced the number of nodules, dry weight of nodules, yield components, grain yield components, nutrient availability and uptake in soybean crop (Son *et al.,* 2008), Mohammadi., 2011). Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum*, while co-inoculation of PSM and PGPR reduced P application by 50% without affecting maize yield,

while the application of the same bacteria *mycorrhizae* (Yazdani *et al.,* 2009) led to the increment maximum grain weight (Mehrvars *et al.,* 2008).

Phosphorus solubilizing bacteria mainly *Bacillus, Psedomonas* and *Enterobacter* are very effective for increasing the plant available P in the soil as well as the growth and yield of crops.

Consequently, exploitation of phosphate solubilizing bacteria through biofertilization has enormous rate of fixing P in the soil, and natural reserves of phosphate rocks.

2.8 Ultisols and their Physical Characteristics

Ultisols is gotten from Latin *ultimus*, "last". These are strongly leached, acid forest soils with relatively low native fertility. They are primarily found in humid temperature and tropical areas of the world typically on older, stable landscapes (Geissen *et al..,* 2009). Intense weathering of primary minerals has occurred and much Ca, Mg and K has been leached from the soils (Girmay *et al.,* 2009) because of favorite climate regimes in which they are typically located, Ultisols often support forests (Gyssels and Poesen, 2003).

Ultisols are low-nutrient and nutrient bearing mineral contents. Typical characteristics of ultisols include:

- Ultisols form through the processes of clay, mineral weathering. Clays with the possibility of oxides, accumulate in the B-
- subsurface horizon. Ultisols are not as widely weathered as Oxisols.
- Ultisols usually develop in warm, moist climates on old land.
- The B horizon may be slightly acidic. The CEC is saturated with less than 35% base-cations.

- Unlike Mollisols, the clay in Ultisols considered to be low activity, or low fertility. However, Ultisols usually contain clay that is not sticky and are very workable.

2.9 Fate of Applied Phosphorus in Ultisols

Ultisols occupy a wide area globally which is about 25.9% of the total geographical area. (Sharma and Sakar, 2005). In humid regions rainfall causes excessive leaching of Phosphorus in ultisols making it the most limiting nutrient for crop production in the soils. Ultisols are weathered acid tropical soils which have high phosphorus sorption capacity (NRCS, 2014). Under normal conditions, most of the phosphorus added through the application of fertilizers is water soluble and readily available to plants. Buts in the case of ultisols, applied phosphorus gradually reacts with Fe and Al compounds and clay minerals present in the soil which consistently gets it transformed into relatively insoluble compounds which are hardly available to plants (Brandy and Weil, 2002). There will be a continuous negative imbalance P balance in ultisols because of the adverse characteristics of the soil (Nwoke *et al.,* 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in the Faculty of Agriculture Research Field, University of Benin. The area has two major seasons namely, the rainy season (April to October) and the dry season (November to March). The mean average daily temperature is 2500mm. The study lies within latitude 6º 24'0"N and 6º 24'10"N and longitude 5º 37'20"E and 5º 37'30"E with an altitude range of 16.2m above sea level with a mean annual rainfall of 26.5^0c. The predominant vegetation of the area is grass with Panicum maximum being the main weed.

3. 2 SAMPLING PROCRDURES

Soil samples were obtained with the use of a soil auger at random in the practical site and at the depths of 0-15cm. The soil samples were air dried and sieved with a 2mm sieve after which they were bagged and properly labelled for physical, chemical and biological analysis.

3.2.1 FIELD PREPARATION

-PRE-PLANTING AND PLANTING OPERATIONS

The field was cleared with the aid of a cutlass and hoe and the weeds were left so as to conserve nutrients. Types of weeds found were Chromolena odorata (Siam weed), Mimosa spp(Sensitive plant), Panicum maximum(Guinea grass).

-Experimental Design: The experiment was laid out in a randomized complete block design (RCBD) with three treatment replicated three times and the treatments were: T_1-Control, T_2-NPK

15:15:15 ammended plots and T_3-NPK+ PSB. Where each plot measured 2.5m x 2.5m and the total area cultivated was 56.25m^2.

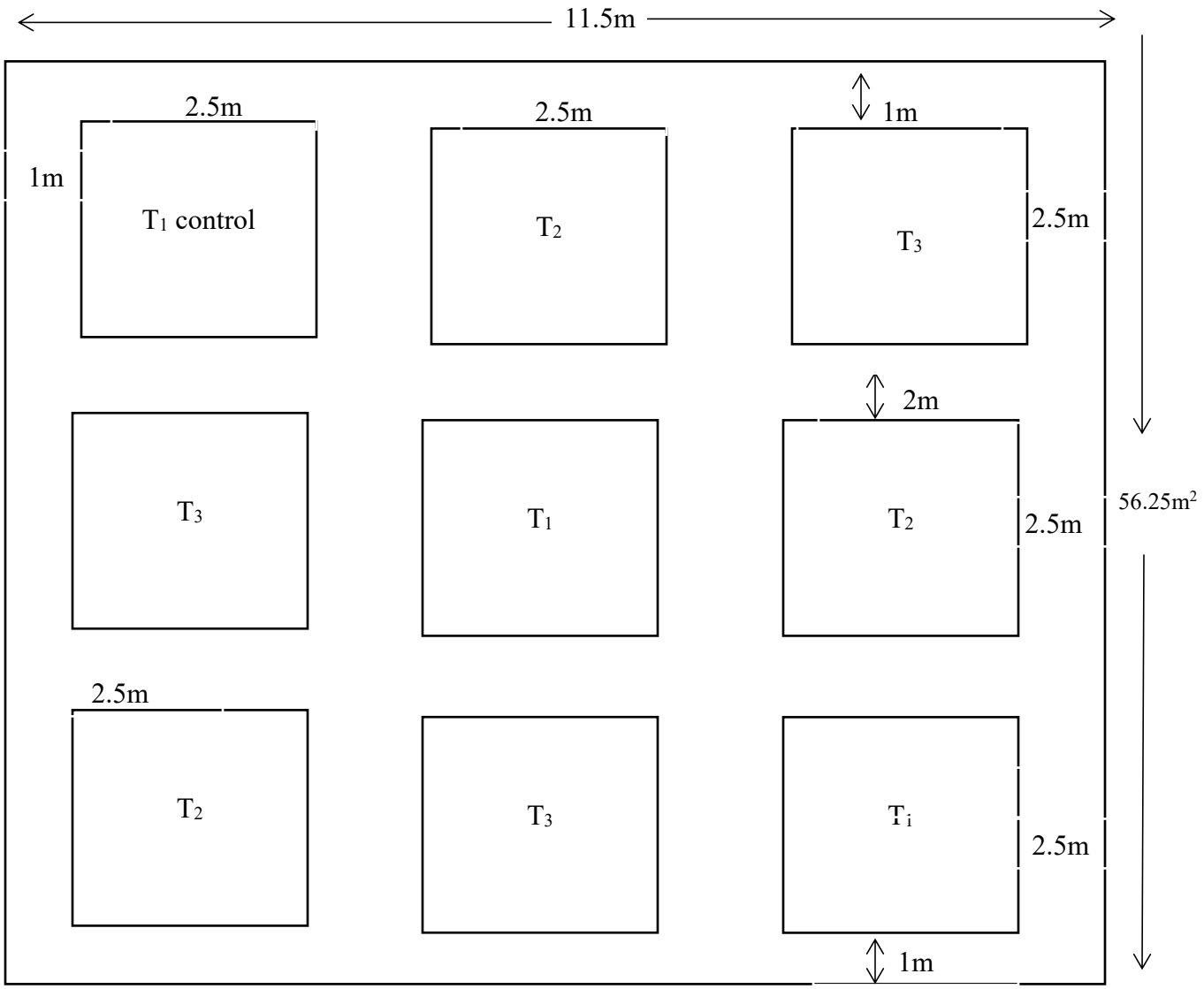

FIGURE 1: Farm Layout

SOWING: The seeds were sown on the 6th of April, 2021 with 2 seeds per hole which were thinned at 2 weeks to the plant with the best vigor. The total number of plants per plot was twelve (12). The name and variety of the sown seeds was the maize hybrid seed (Oba 98).

HARVESTING: This was done when the combs were dried and they were dehusked. The weight of maize per treatment was ascertained and 1000 grain weight was also determined after the initial weighing.

3.2.2 MEASUREMENT OF AGRONOMIC PARAMETERS

The first parameter readings were taken 2 weeks after sowing. Subsequent readings were taken at the 4th, 6th and 8th weeks respectively. The number of leaves were counted, the plant height was measured with a meter rule with accurate readings. The leaf length was determined by placing the most developed leaf on the meter rule. Calculations were taken gotten through the multiplication of the leaf length by the leaf width with the constant 0.75 (i.e leaf area = leaf length x leaf width x 0.75).

The stem girth was measured by using a twine around the girth, holding it tightly and placing it on a meter rule for results.

POST-PLANTING: Weeding was regularly done through handpicking and the use of hand hoes to remove the weeds. The weeds were carefully returned back to the soil to conserve and return the nutrients to the soil. They also served as mulch materials.

NPK APPLICATION: Application of $NPK_{15:15:15}$ fertilizer at the rate of 34.72g per plant was done by ring method.

3.3 PHYSICAL AND CHEMICAL ANALYSIS OF SOIL

3.3.1 PARTICLE SIZE DETERMINATION

Air dried and sieved soil sample was analyzed for particle size using the Bouyoucos hydrometer method (Gee and Or, 2002). 51g of air dried soil was weighed and placed in a soil shaking bottle, 100ml of sodium hexametaphosphate was added into the beaker containing the 51g of soil. Stirring of solution was gently done and it was poured into the beaker containing the 51g of soil. Stirring of the solution was gently done and it was poured into a 1000ml calibrated measuring cylinder. Distilled water was added up to the 1000ml mark. Three drops of hydrogen peroxide was added. The solution was set in motion. It was then left to settle for 40 seconds with the density measured immediately with the hydrometer, then the density and temperature readings were taken again using the hydrometer.

Calculation

$$\% \text{ Sand} = \frac{\text{corrected 40 seconds reading}}{\text{weight of sample taken}} \text{ x } 100$$

$$\% \text{ clay} = \frac{\text{corrected 2 hours reading}}{\text{weight of sample taken}} \text{ x } 100$$

% silt = 100- (% sand + clay).

The results were expressed in gkg^{-1} through the multiplication of the percentage quantities by 10.

3.3.2 HYDROGEN ION CONCENTRATION

The pH was obtained using a pH meter (Hendershot *et al.,* 1993). 50ml beaker was placed on the weighing balance and it was set at point 0. 10g of soil sample was weighed using the weighing balance and placed in a soil shaking bottle. 10g of water was measured using measuring cylinder.

It was added to the 10g of soil sample to form solution. The solution was stirred intermittently for about 30 minutes and it was allowed to rest for 5 minutes after stirring. A standardized pH meter was used to take the pH reading of the solution in water. 0.01m of $Cacl_2$ was added to the initial soil solution of water and stirred for 15 minutes. The pH solution was taken and the reading was recorded.

3.3.3 SOIL ORGANIC CARBON DETERMINATION

Soil organic carbon was determined by Walkey-Black method (Nelson and Sommer 1996). 1g of soil was weighed into 250ml conical flask where 10ml of potassium dichromate was added ($\mathbf{k_2Cr_2O_7}$) was added. Concentrated H_2SO_4 measuring 15ml was added carefully to avoid spillage.

After oxidation, 100ml of water was added followed by 5 drops of Ferroin indicator. Titration of the solution against 0.5ml of $FeSO_4$ was carried out swirling at intervals until the mixture was taken.

Calculation of organic carbon

$$\frac{(\mathrm{CB} - \mathrm{T})\ \mathrm{x}\ \mathrm{M}\ \mathrm{x}\ 0.003\mathrm{F}}{\mathrm{W}}\ \mathrm{x}\ 100$$

B = Black titre value

T = Sample titre value

M = Molarity of concentrated $FeSO_4$

F = Correlation factor 1.33

3.3.4 TOTAL NITROGEN DETERMINATION

Total nitrogen (TN) was determined by Kjeldahl (Bremmer, 1996). 5g of soil was weighed into a soil shaking bottle. 25ml of NH_4^- (extracting method) was added into the shaking bottle containing the soil. The solution was shaken continuously for 30 minutes after which the solution was filtered into a conical flask with a filter paper. The filtrate was turned into a 10ml measuring cylinder to get to 5ml. The 5ml filtrate was added in added in ratio 2:2:1 and filled with water to get to 5ml. The beaker was covered and solution shaken gently to avoid it from spilling. The solution was then put in a curvette and the curvette was inserted into the spectrometer to take reading.

Calculation

$$\text{PPM or Mg\%} = \frac{\text{IR x SR x colour developer x E. R}}{W} \text{ x Aliquot}$$

Where IR = Instrument reading = 0.085

SR = Slope reciprocal

Colour developer = 10ml

Extracting reagent = 20ml

W= 5g

Aliquot = 5ml

3.3.5 EXCHANGEABLE BASES

Using the EDTA titration procedure, 10g of air dried soil was weighed into a 250ml soil shaking bottle. 100ml of 1N Ammonium acetate was added and was shaken in a mechanical shaker for 1

hour. The soil was filtered using a filter paper into a 100ml volumetric flask and made up to 100ml with ammonium acetate solution. Potassium and sodium were read in a flame photometer and calcium and magnesium were read in an atomic absorption spectrophotometer.

3.3.6 EXCHANGEABLE ACIDITY

10g of soil sample was shaken with 100ml 1N KCl for 1 hour on a reciprocal shaker and thereafter filtered through filter paper. The aliquot was titrated with 0.1N NaOH using 4 drops of phenolphthalein indicator to a pink end point. Thereafter results were expressed as $Cmolkg^{-1}$.

3.4 ENUMERATION AND IDENTIFICATION OF TOTAL VIABLE BACTERIA

3.4.1 CULTURE MEDIA PREPARTION

Culture media used was nutrient agar (NA) or the culture of Bacteria and MacConkey agar for sub culturing. While preparing the media, 28g of the nutrient agar was weighed into a 250ml Erlenmeyer flask and dissolved with 100ml of distilled water. This was sterilized using an autoclave for 120^0c for 15 minutes.

GLASSWARES

1. Disposable petri-dishes/plates
2. Test tubes and test tube racks
3. Conical flask and beakers
4. Mac Cartney bottles and Durhams Spirit lamps and disinfectants
5. Slides
6. Pipette
7. Bijoux bottles

Sterilizing materials and measures

I. Autoclave

II. Bunsen burner

III. Oven

All glass wares were properly washed with water, disinfectants and brushes were sterilized in air oven at 60^0c for 24 hours to achieve maximum sterilization.

Other materials

1. Microscope
2. Whitman filter paper
3. Peptone water
4. Distilled water
5. Incubator
6. Sterile swab stick
7. Cotton wool and aluminum foil paper
8. Sterilized blender

Preparation of Sample for culture/ serial dilution

10 fold serial dilution was made for each sample. 10 test tubes were filled with 9ml of sterile distilled water. 1g of the soil was weighed out and transferred into 9ml test tube and thoroughly mixed.1ml of the 10ml mixture was transferred by pipetting to the 9ml diluents in the other test tubes for up to 10 test tubes.

3.6 DETERMINATION OF MICROBIAL ISOLATES FROM SOIL SAMPLES

After serial dilution, “pour plate method” dilutions of 10^5 were prepared for each of the soil samples.

Bacteria: A Pour plate of the serial were prepared by using approximately 20ml of nutrient agar, amended with a mixture and 0.5ml of serial suspension from each of the soil samples

3.7 Plate count

The bio load of the microorganisms is expressed in colony forming unit (cfu) as described by (Daniel, 2001). The agar plates having the culture colonies after incubation were divided into four and two vertically opposite sections were counted and multiplied by two. This was multiplied again by the dilution factor and gave the colony forming unit of the sample. Bacteria isolates were identified by standard microbial techniques as described by Gram stain.

Gram stain: It was used to differentiate G+ bacteria by means of difference in their cell wall composition.

Procedure: Smears of all isolates were made on sterile distilled water. The smears were air dried and heat fixed. Each of the slides were flooded with crystal violet for 1 minute; washed with water, stained with iodine acting as a mordant. The slide was washed again with water, decolorized with 90% alcohol until the run off was clear, then it was counter-stained with safarin for 15 seconds. Finally, the slide was rinsed with water. This was observed under the high power microscope under oil immersion

3.7 BIOCHEMICAL TESTS

Sugar fermentation test: This test was used to demonstrate the ability of some bacteria to utilize some sugars as substrates. The sugar substrates used were glucose, lactose, mannitol. 10g of wach test sugar was added to 1 litre peptone water and 5% of 0.2% phenol red solution (5ml of 0.2% phenol red solution of 95ml of sample) was added as indicator. The medium was dispensed into Mac Cartney bottles containing Durham tubes to a depth of about 4cm. The sugars allowed to cool down before inoculation was made with the test isolates (except one which was used as control) and incubated aerobically at 37^0c for about 48hours. Yellow colorations indicated acid production and thus a positive result and presence of gas in the inverted Durham tubes indicated gas production.

i) Oxidate test

A piece of filter paper was placed on a clean petridish with 2 to 3 drops of oxidase added to soak the filter paper. A sterile glass rod was used to collect test organisms to mark the smear of it on the paper. An appearance of a deep purple within 10 seconds indicates a positive reaction.

ii) Citrate test

This test is used to ascertain the ability of an organism to use citrate as its source of carbon. The test organism was aceptically inoculated using smear method and streak technique on sterile Simmon's citrate agar slants in Bijoux bottle and butt stabbed. The slants were incubated for 48 hours at 53^0C to look for colour change of the culture medium from green to bright blue indicates positive citrate utilization.

iii) Indole test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffed peptone water. The test is usually used as an aid in the differentiation of gram negative *Bacilli* especially those Enterobacteriace. Bijoux bottles containing peptone water was aseptically inoculated with young culture of the test organism isolates. It was inoculated for about 48 hours. Then 0.5ml of Kovac's reagent was added to 1ml of each medium gently shaking and allowed to stand for 5 minutes. Purple or deep red coloration was recorded as positive indole production test. Yellow or no colour change was recorded as negative indole production test.

iv) Methyl red test

This is used in the detection of acid production. 10ml bottle of MRVP medium was aseptically inoculated with two loopful of pure 24 hours peptone water culture of the test organisms. The bottle was inoculated for 48 hours at 37^0C. Thereafter, 5 drops of methyl red reagent was added to one of each of the culture. Bright red colour indicated negative result as in the production of indole test.

3.8 STATISTICAL ANALYSIS

Data obtained from the experiment were subjected to analysis of variance (ANOVA) using the 8^{th} edition of GENSAT statistical package and Duncan Multiple range test was used to separate mean at 5% level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 INITIAL PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL SAMPLES

The physical and chemical properties of the soil used for the study are presented in Table 1. The soil was slightly acidic (pH of 4.0). The acidic nature of the soil could be due to the leaching of basic cations such as Ca and Mg in the soil. The soil was also discovered to be low (9.1 kg^{-1}) in Organic Carbon content compared with the critical value of 20gkg^{-1} as defined by Adauyi *et al.,* (2002). The soil total nitrogen (0.93gkg^{-1}) was also observed to be lower than the critical value of 1.5gkg^{-1} (Adauyi *et al.,* 2002). The low total N could be attributed to the high rate of mineralization. The same trend was recorded with the soil available Phosphorus which has a value of 1.01mg/kg. The low exchangeable bases (ca, mg, k and Na) coupled with the low primary (NPK) nutrients suggest that the soil is low in fertility and would therefore need ammendments (inorganic or organic) for a proper production capacity.

Table 1: Initial Physical and Chemical properties of the soil sample

PARAMETERS	UNITS	RESULTS
Sand	*g/kg*	880
Silt	*g/kg*	60
Clay	*g/kg*	60
Texture		Loamy sand
pH (1:2)	H_20	4.0
Cond.	*µs/cm*	62.90
Org. C	*g/kg*	9.1
Org.M	*g/kg*	15.69
Total N	*g/kg*	0.93
Ca	*cmol/kg*	0.78
Mg	*cmol/kg*	0.80
Na	*cmol/kg*	0.22
K	*cmol/kg*	0.13
CEC	*cmol/kg*	1.93
ECEC	*cmol/kg*	2.33
Avail.P	*mg/kg*	1.01
Un Av. P	*mg/kg*	15.73
Sulphate-S	*mg/kg*	469.80
Total Fe	*mg/kg*	999.94
H	*cmol/kg*	0.10
Al	*cmol/kg*	0.30

PLATE 1: ACTIVITY PHOTOS

4.2 PLANT PARAMETER READINGS

The plant parameters are presented in table 2.1. There were no significant differences for the plant indices (Number of leaves, leaf area, stem girth) in week 2 as no inorganic amendment have been applied. NPK fertilizer was applied at week 4 with T_2 and T_3 having NPK $_{15:15:15}$ as a constituent treatment there was significant increase for T2 having the (number of leaves, plant height, leaf area and stem girth at 8, 42.67cm,192.7cm^2 and 5cm) respectively even though it was the lowest at week 4 and it could be attributed to the leaching of the nutrient elements in NPK $_{15:15:15}$ which is in line with investigations made by Bhattacharjee *et al* 2008 and T_3 had the second highest plant indices at week 4. T_1 had the highest plant parameter indices at week 4 and this could be attributed to better mulch materials according to Akanbi and Ojeniyi (2007). Week 6 saw T_3 having the highest maize plant at 110.0cm. Observable changes occurred especially when T3 was inoculated with *Bacillus sp* after week 8. There was a significant increase in the plant parameters (having the highest plant and widest leaf area) as compared to week 6 when it had the second highest after T_1. This indicates that *Bacillus sp* was effective and its effectiveness contributed to increase in maize production.

4.3 MAIZE GRAIN YIELD (1000 grains)

The maize grain yield results are presented in Figure 2.0. It was observed that the NPK+PSB produced the highest grain yields at 8.7g/ 18.75m^2. This could be in line with the inoculation of *Bacillus sp.* NPK produced the second highest grain yield at 7.2g/18.75m^2 compared to Control which had the lowest maize yield (6.6g / 18.75m^2).

Tables 2: Plant parameters as at week 2 to week 8

Treatment	Number of leaves	Plant height	Leaf Area	Stem girth
		Week 2		
T1	5ab	17.10a	35.80a	2a
T2	5a	15.98a	34.77a	2a
T3	6b	18.28a	37.93a	3a
		Week 4		
T1	10	69.336	266.2a	7a
T2	8	42.67a	192.7a	5a
T3	9	51.10ab	211.0a	6a
		Week 6		
T1	13b	99.3a	600.7	7a
T2	10a	93.3a	368.3a	6a
T3	11ab	111.0a	412.3a	7a
		Week 8		
T1	11a	135.7a	350.7a	7b
T2	11a	118.7a	420.3ab	6a
T3	10a	120.0a	509.0b	6ab

Means with the same letters(s) are not significantly different from each other at $p< 0.05$

T1= Soil (Control)

T2= NPK

T3= NPK + *Bacillus sp.*

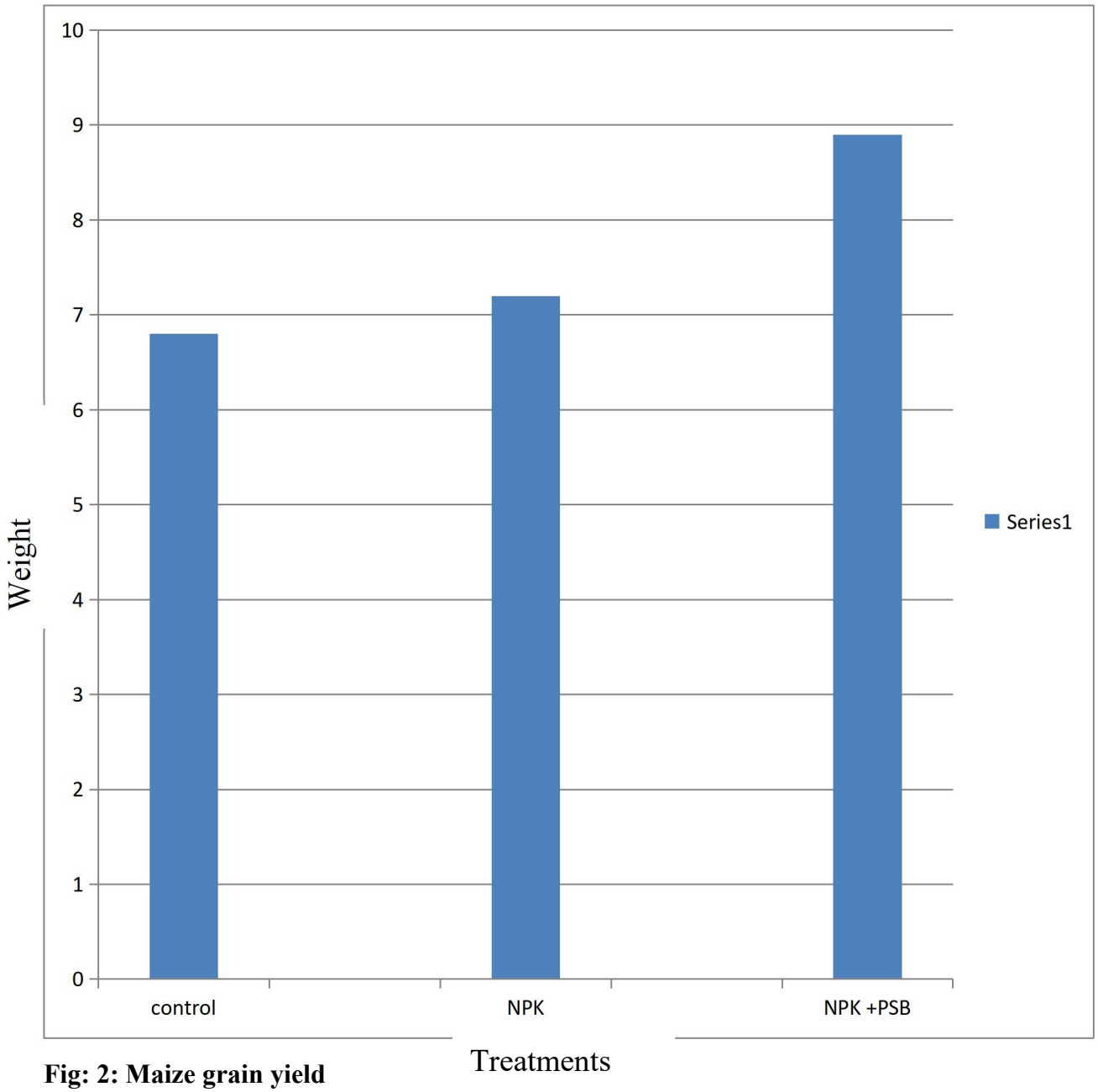

Fig: 2: Maize grain yield

- **Control (T_1)**
- **NPK$_{15:15:15}$ (T_1)**
- **NPK $_{15:15:15}$ + *Bacillus sp***

4.4 FINAL PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL

Effects of the soil amendment (NPK $_{15:15:15}$) inoculum (*Bacillus sp*) are presented in table 3.1. The results from the table showed that the application of NPK $_{15:15:15}$ inoculum (*Bacillus sp*) significantly improved the soil physical and chemical properties. The initial pH result showed that it was slightly acidic at 4.0(in table 4.1). The final result at T_1(Control), T_2(NPK), T_3(NPK+ *Bacillus sp*) were 4.6, 4.7 and 4.6 respectively as strongly acidic soils according to Chude *et al.,* 2011). The organic carbon level for T_1, T_2, T_3 were 7.4g0kg^{-1}, 9.55gkg^{-1} and 9.60gkg^{-1}. It was observed that the rates were below the critical level of 20gkg^{-1} as defined by Adauyi *et al.,*2002. The low organic carbon levels could be to the reduced presence of decaying organisms in the soil. Soil total N for the 3 treatments were (0.06gkg^{-1},0.86gkg^{-1} and 0.87gkg^{-1}). The total N was low for the 3 treatments as they were below the critical value of 1.5gkg^{-1}. Despite the fact that the treatments (T_2 and T_3) received NPK fertilizers, the total N was still low. This could be due to the high level of leaching of the nutrients in the soil accompanied with the heavy rains. Initial available P rate was 1.01mg/kg. Available P increased from that initial rate of 1.01mg/kg to 8.65mg/kg, 19.42mg/kg and 20.01mg/kg for the 3 treatments. It was seen that T_3 had the highest rate of 20.01mg/kg as it was above the critical level of 20mg/kg. This could be due to the fact that the addition of NPK and inoculum (*Bacillus sp*).

4.5 BACTERIA POPULATION COUNT FOR THE DIFFERENT TREATMENTS

Figure 3 shows the bacteria population in the soil. From the result, bacteria count in T_1 was 9 x10^{3}cfu/ml, T_2(NPK) was 4 x10^{3}cfu/mg and T_3(NPK+ *Bacillus sp*) was 7x 10^{3}cfu/ml. From the result, it was observed that T_1(control) had the highest bacteria population and T_1 did not receive any form of soil amendment. This could be due to the high level of decomposition and adaptation over time may have made the microorganisms to remain. The most dominant species

were *Staphylococcus aureus*, *Pseudomonas*, *Bacillus sp* and *E. coli*. For T_2 having the least bacteria population, the nutrient supplied (NPK) by the fertilizer is not enough and might have slowed the growth of the organisms. The inoculated treatment having the population of 7x 10^3cfu/ml with the dominant species being *Staphylococcus*, *Bacillus sp* and *E. coli.* The moderate population count could be due to the immobilization of the microorganisms.

Table 3: Final physical and chemical property of the soil

Treatment	*Sand*	*Silt*	*Clay*	*Texture*	*pH(1:2)*	*Cond.*	*Organ. C*	*Organ. M*	*Total N*	*Ca*	*Mg*	*Na*	K
	g/kg	*g/kg*	*g/kg*		*H_2O*	*µs/cm*	*g/kg*	*g/kg*	*g/kg*	*cmol/kg*	*cmol/kg*	*Cmol/kg*	*cmol/kg*
T_1	830	60	110	Loamy	4.6	45.75	7.40	12.9	0.06	0.80	0.06	0.08	0.22
T_2	875	35	90	Loamy	4.7	59.26	9.55	16.55	0.86	1.31	0.10	0.12	0.26
T_3	850	60	90	Loamy	4.6	45.30	9.60	16.75	0.87	1.16	0.18	0.06	0.16

Treatment	*CEC*	*ECEC*	*Avail. P mg/kg*	*Total P mg/kg*	*Total Fe mg/kg*	*SO_4 mg/kg*	*Excan. Acidity cmol/kg*
T_1	1.16	2.93	8.65	254.63	564.00	181.00	1.77
T_2	1.79	2.88	19.42	427.79	640.96	147.50	1.09
T_3	1.56	2.60	20.01	620.82	582.90	356.78	1.04

*T_1(Control)

T_2(NPK)

T_3(NPK+*Bacillus sp*)

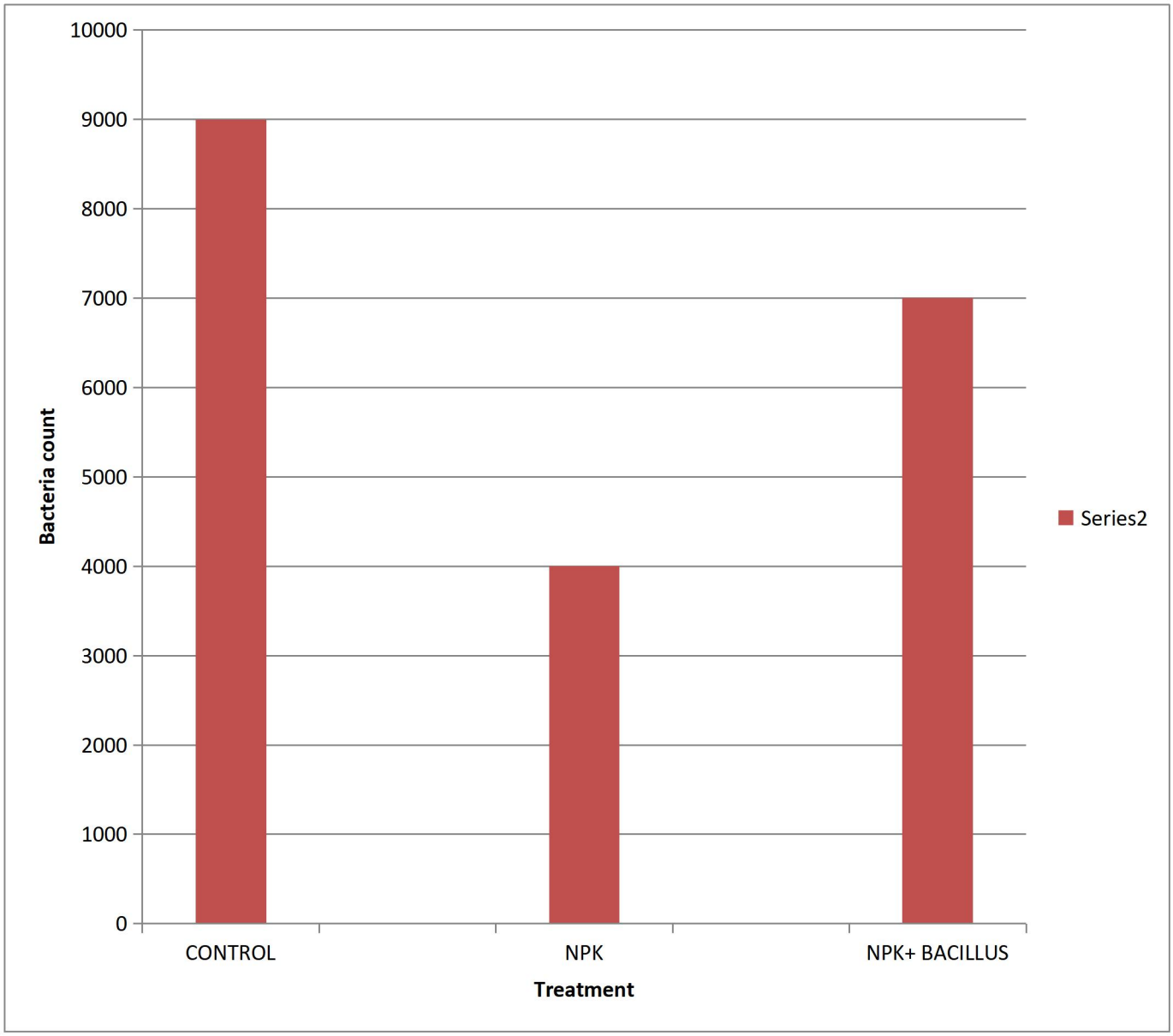

Fig 3: Bacteria Population count for the different treatment

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

The study revealed that the application of T_2 (NPK) and T_3(NPK+ *Bacillus sp*) as soil amendment and inoculum for the T_3 significantly led to the increment of the soil's physical, chemical and vegetative growth rate of the test plant(maize). The leaf area at week 8 was the highest in T_2 and T_3 as against T_1(control). The utilization of NPK with *Bacillus sp*(PSB) greatly enhanced the uptake of plant insoluble P. Presence of PSB also increased the P availability in the rhizosphere of maize as well as the organic matter in composition in the soil. Consequently, the phosphatase activity was enhanced via the presence of PSB.

In line with this study, it is recommended that the residual effect of NPK and *Bacillus sp* should be checked. Moreover, inoculating with Phosphorus Solubilizing Bacteria is a promising avenue in the Agricultural sector in Nigeria and more emphasis should be placed on it.

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APPENDIX

GenStat Release 8.1 (PC/Windows) 22 June 2021 11:04:52
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GenStat Eighth Edition
GenStat Procedure Library Release PL16

Analysis of variance

Variate: no_leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.0956	0.0478	0.43	
replicate.*Units* stratum					
treatments	2	1.1460	0.5730	5.21	0.077
Residual	4	0.4400	0.1100		
Total	8	1.6816			

Tables of means

Variate: no_leaves
Grand mean 5.35

treatments	T1 (control)	T2	T3
	5.43	4.88	5.75

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.191

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.271

All pairwise comparisons are tested.
Variance = 0.1100 with 4 degrees of freedom
Duncan's multiple range test
Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776	
3	0.9025	2.837	

Mean	vs Mean	t	significant
T2	T1 (control	-2.031	No
T2	T3	-3.188	Yes
T1 (control	T3	-1.157	No

Identifier Mean

T2	4.883	|
T1 (control	5.433	| |
T3	5.747	|

Analysis of variance

Variate: plant_ht

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	9.625	4.812	0.59	
replicate.*Units* stratum					
treatments	2	7.960	3.980	0.49	0.647
Residual	4	32.727	8.182		
Total	8	50.312			

Tables of means

Variate: plant_ht

Grand mean 17.12

treatments	T1 (control)	T2	T3
	17.10	15.98	18.28

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	1.651

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	2.336

All pairwise comparisons are tested.

Variance = 8.1818 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500

Comparisonwise error rates

2	0.9500	2.776	
3	0.9025	2.837	
Mean	vs Mean	t	significant
T2	T1 (control	-0.4796	No
T2	T3	-0.9862	No
T1 (control	T3	-0.5067	No

Identifier	Mean	
T2	15.98	|
T1 (control	17.10	|
T3	18.28	|

Analysis of variance

Variate: leaf_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	79.15	39.58	0.45	
replicate.*Units* stratum					
treatments	2	15.65	7.82	0.09	0.917
Residual	4	351.71	87.93		
Total	8	446.51			

Tables of means

Variate: leaf_area

Grand mean 36.2

treatments	T1 (control)	T2	T3
	35.8	34.8	37.9

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	5.41

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	7.66

All pairwise comparisons are tested.
Variance = 87.9265 with 4 degrees of freedom

Duncan's multiple range test
Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776	
3	0.9025	2.837	
Mean	vs Mean	t	significant
T2	T1 (control	-0.1350	No
T2	T3	-0.4136	No
T1 (control	T3	-0.2786	No

Identifier	Mean	
T2	34.77	|
T1 (control	35.80	|
T3	37.93	|

Analysis of variance

Variate: stem_gt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.9987	0.4993	0.89	
replicate.*Units* stratum					
treatments	2	2.5535	1.2767	2.28	0.218
Residual	4	2.2376	0.5594		
Total	8	5.7898			

Tables of means

Variate: stem_gt
Grand mean 2.71

treatments	T1 (control)	T2	T3
	2.20	2.49	3.45

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.432

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.611

All pairwise comparisons are tested.
Variance = 0.5594 with 4 degrees of freedom
Duncan's multiple range test
Experimentwise error rate = 0.0500
Comparisonwise error rates

	2	0.9500	2.776
3	0.9025	2.837	

Mean	vs Mean	t	significant
T1 (control	T2	-0.475	No
T1 (control	T3	-2.041	No
T2	T3	-1.567	No

Identifier	Mean	
T1 (control	2.200	|
T2	2.490	|
T3	3.447	|

Analysis of variance

Variate: no_leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.6067	0.3033	2.39	
replicate.*Units* stratum					
treatments	2	7.8867	3.9433	31.13	0.004
Residual	4	0.5067	0.1267		
Total	8	9.0000			

Tables of means

Variate: no_leaves

Grand mean 8.90

treatments	T1 (control)	T2	T3
	9.93	7.67	9.10

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.205

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.291

All pairwise comparisons are tested.

Variance = 0.1267 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-4.932	Yes
T2	T1 (control	-7.800	Yes
T3	T1 (control	-2.868	Yes

Identifier	Mean
T2	7.667
T3	9.100
T1 (control	9.933

Analysis of variance

Variate: plant_ht

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	151.01	75.50	1.14	
replicate.*Units* stratum					
treatments	2	1114.69	557.34	8.41	0.037
Residual	4	265.15	66.29		
Total	8	1530.84			

Tables of means

Variate: plant_ht

Grand mean 54.4

treatments	T1 (control)	T2	T3
	69.3	42.7	51.1

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	4.70

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	6.65

All pairwise comparisons are tested.

Variance = 66.2867 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-1.269	No
T2	T1 (control	-4.011	Yes
T3	T1 (control	-2.743	No

Identifier	Mean		
T2	42.67	|	
T3	51.10	|	|
T1 (control	69.33		|

Analysis of variance

Variate: leaf_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	52906.	26453.	2.61	
replicate.*Units* stratum					
treatments	2	11571.	5785.	0.57	0.605
Residual	4	40550.	10138.		
Total	8	105027.			

Tables of means

Variate: leaf_area

Grand mean 243.

treatments	T1 (control)	T2	T3
	266.	193.	271.

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	58.1

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	82.2

All pairwise comparisons are tested.

Variance = 10137.5267 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T1 (control	-0.8949	No
T2	T3	-0.9529	No
T1 (control	T3	-0.0580	No

Identifier	Mean	
T2	192.7	|
T1 (control	266.2	|
T3	271.0	|

Analysis of variance

Variate: stem_gt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	1.6156	0.8078	1.42	
replicate.*Units* stratum					
treatments	2	4.9689	2.4844	4.36	0.099
Residual	4	2.2778	0.5694		
Total	8	8.8622			

Tables of means

Variate: stem_gt

Grand mean 5.96

treatments	T1 (control)	T2	T3
	7.00	5.33	5.53

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.436

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.616

All pairwise comparisons are tested.

Variance = 0.5694 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-0.325	No
T2	T1 (control	-2.705	No
T3	T1 (control	-2.380	No

Identifier	Mean	
T2	5.333	|
T3	5.533	|
T1 (control	7.000	|

Analysis of variance

Variate: no_leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	3.5467	1.7733	1.96	
replicate.*Units* stratum					
treatments	2	11.9467	5.9733	6.59	0.054
Residual	4	3.6267	0.9067		
Total	8	19.1200			

Tables of means

Variate: no_leaves

Grand mean 11.07

treatments	T1 (control)	T2	T3
	12.67	10.00	10.53

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.550

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.777

All pairwise comparisons are tested.

Variance = 0.9067 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-0.686	No
T2	T1 (control	-3.430	Yes
T3	T1 (control	-2.744	No

Identifier	Mean		
T2	10.00	|	
T3	10.53	|	|
T1 (control	12.67		|

Analysis of variance

Variate: plant_ht

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	494.2	247.1	0.87	
replicate.*Units* stratum					
treatments	2	484.2	242.1	0.86	0.490
Residual	4	1131.1	282.8		
Total	8	2109.6			

Tables of means

Variate: plant_ht

Grand mean 101.2

treatments	T1 (control)	T2	T3
	99.3	93.3	111.0

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	9.71

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	13.73

All pairwise comparisons are tested.

Variance = 282.7778 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T1 (control	-0.4370	No
T2	T3	-1.2867	No
T1 (control	T3	-0.8497	No

Identifier	Mean	
T2	93.3	|
T1 (control	99.3	|
T3	111.0	|

Analysis of variance

Variate: leaf_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	5583.	2791.	1.71	
replicate.*Units* stratum					
treatments	2	91384.	45692.	27.97	0.004

Residual	4	6535.	1634.
Total	8	103502.	

Tables of means

Variate: leaf_area

Grand mean 460.

treatments	T1 (control)	T2	T3
	601.	368.	412.

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	23.3

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	33.0

All pairwise comparisons are tested.

Variance = 1633.7778 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-1.333	No
T2	T1 (control	-7.040	Yes
T3	T1 (control	-5.707	Yes

Identifier	Mean	
T2	368.3	|
T3	412.3	|
T1 (control	600.7	

Analysis of variance

Variate: stem_gt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

replicate stratum	2	1.5556	0.7778	2.80	
replicate.*Units* stratum					
treatments	2	1.5556	0.7778	2.80	0.174
Residual	4	1.1111	0.2778		
Total	8	4.2222			

Tables of means

Variate: stem_gt

Grand mean 6.56

treatments	T1 (control)	T2	T3
	6.67	6.00	7.00

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.304

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.430

All pairwise comparisons are tested.

Variance = 0.2778 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T1 (control	-1.549	No
T2	T3	-2.324	No
T1 (control	T3	-0.775	No

Identifier	Mean	
T2	6.000	|
T1 (control	6.667	|
T3	7.000	|

Analysis of variance

Variate: no_leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	0.762	0.381	0.22	
replicate.*Units* stratum					
treatments	2	0.309	0.154	0.09	0.916
Residual	4	6.858	1.714		
Total	8	7.929			

Tables of means

Variate: no_leaves

Grand mean 10.59

treatments	T1 (control)	T2	T3
	10.67	10.77	10.33

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.756

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	1.069

All pairwise comparisons are tested.

Variance = 1.7144 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T3	T1 (control	-0.3118	No
T3	T2	-0.4053	No
T1 (control	T2	-0.0935	No

Identifier	Mean	
T3	10.33	|
T1 (control	10.67	|

T2 10.77 |

Analysis of variance

Variate: plant_ht

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	1020.2	510.1	1.93	
replicate.*Units* stratum					
treatments	2	536.2	268.1	1.02	0.440
Residual	4	1055.1	263.8		
Total	8	2611.6			

Tables of means

Variate: plant_ht

Grand mean 124.8

treatments	T1 (control)	T2	T3
	135.7	118.7	120.0

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	9.38

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	13.26

All pairwise comparisons are tested.

Variance = 263.7778 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-0.1005	No
T2	T1 (control	-1.2820	No
T3	T1 (control	-1.1814	No

Identifier	Mean	
T2	118.7	|
T3	120.0	|
T1 (control	135.7	|

Analysis of variance

Variate: leaf_area

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	10656.	5328.	1.50	
replicate.*Units* stratum					
treatments	2	37785.	18892.	5.32	0.075
Residual	4	14197.	3549.		
Total	8	62638.			

Tables of means

Variate: leaf_area

Grand mean 427.

treatments	T1 (control)	T2	T3
	351.	420.	509.

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	34.4

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	48.6

All pairwise comparisons are tested.

Variance = 3549.3333 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T1 (control	T2	-1.432	No
T1 (control	T3	-3.255	Yes

T2	T3	-1.823	No

Identifier	Mean		
T1 (control	350.7	|	
T2	420.3	|	|
T3	509.0		|

Analysis of variance

Variate: stem_gt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicate stratum	2	1.3867	0.6933	5.47	
replicate.*Units* stratum					
treatments	2	1.1467	0.5733	4.53	0.094
Residual	4	0.5067	0.1267		
Total	8	3.0400			

Tables of means

Variate: stem_gt

Grand mean 6.27

treatments	T1 (control)	T2	T3
	6.67	5.80	6.33

Standard errors of means

Table	treatments
rep.	3
d.f.	4
e.s.e.	0.205

Standard errors of differences of means

Table	treatments
rep.	3
d.f.	4
s.e.d.	0.291

All pairwise comparisons are tested.

Variance = 0.1267 with 4 degrees of freedom

Duncan's multiple range test

Experimentwise error rate = 0.0500
Comparisonwise error rates

2	0.9500	2.776
3	0.9025	2.837

Mean	vs Mean	t	significant
T2	T3	-1.835	No
T2	T1 (control	-2.982	Yes
T3	T1 (control	-1.147	No

Identifier	Mean		
T2	5.800	|	
T3	6.333	|	|
T1 (control	6.667		|