CHAPTER ONE

1.0 INTRODUCTION

Maize was introduced to Africa in the 1500s and has since become one of Africa's dominant food crops. It is the most important cereal crop in sub-Saharan Africa (SSA), West and Central Africa and currently accounting for a little over 20% of the domestic food production in Africa (Smith *et al*., 1994). Its importance has increased as it has replaced other food staples, particularly sorghum and millet (Smith *et al*., 1997). Trends in maize production indicate a steady growth, mostly due to the expansion of the cultivated area, and also the result of improved maize yields. In 1989-1991, the average maize yield in Africa is 1.2 tonnes per hectare, it was twice that estimated in the 1950s this is because improved varieties are now generally available (Byerlee and Eicher, 1997).

Maize, which was traditionally grown as a subsistence crop on small plots in home gardens, has been transformed into a commercial and profitable crop in the farming systems of different agro-ecological zones of West and Central Africa. In Nigeria, its production is quite common in all parts of the country, from the north to the south, with an annual production of about 5.6 million tones (Central Bank of Nigeria, 1992). The country's maize crop covers about 1million hectare out of 9 million hectares it occupied in Africa (Hartmans, 1985). Per capital maize consumption has been growing at rate of 0.3% annually; 1983 - 1992 in West Africa (Adesina *et al*., 1997). Studies on maize production in different part of Nigeria have shown an increasing importance of the crop amidst growing utilization by food processing industries and livestock feedmills. The crop has thus grown to be local 'cash' most especially in the southwestern part of Nigeria where at least 30% of the crop land has been devoted to maize production under various cropping system (Alabi and Onolemhemhen, 2001).

About 80% of maize produced in West and Central Africa is used in the preparation of foods, animal feed and fodder. Industrially processed foods such as corn flours, corn flakes, maize syrups, soft drinks, breakfast cereals, starch, and e.t.c are processed from maize. Stem and foliage of the plant are used for hay, silage, and pasture (Adesina *et al*., 1997). Maize can as well be prepared into various food items like pap, 'tuwo', 'gwate', 'donkunu' and host of others. All these food types are readily available in various parts of Nigeria among different ethnic groups, notably among the Yorubas, Hausas, Ibos, Ibiras, Ishas, Binis, Efiks, and Yalas. Preparations and uses of the maize grains varied from group to group, though at time with some similarities. Apart

from food, maize is also useful as medicines and as raw materials for industries (Abdulrahaman and Kolawole, 2006).

Ironically, the demand for maize in Nigeria for various industrial and domestic uses sometimes outstrips supply (Ogunwale *et al*., 1998; Anyanwu *et al.*, 2005). The production is often low in Nigeria owing to combination of agro-biological (IITA, 2009), climatic (Agbola and Ojeleye, 2007; CIMMYT, 2010) and technological factors (Ojo, 2000; Iken and Amusa, 2004) coupled with high post-harvest losses (Agoda *et al*., 2011). In addition to continued soil nutrient depletion (Kang, 1981; Iken and Amusa, 2004), diseases such as downy mildew, rust, leaf blight, stalk and ear rots, leaf spot, maize streak virus, seedling root rot, stalk rot, and collar rot of seedlings (White, 2000; Andrés-Ares *et al*., 2004) are among the major factors that limit maize production in Southwestern Nigeria. With respect to this, efforts are made to increase maize production through maize seed multiplication and development of high yielding disease-resistant varieties that are adaptable to various agro-ecological zones (IITA, 2009). The Agricultural Development Projects often assist the out-growers by providing fertilizers and other production inputs (Roy, 2006). However, despite all these agro-technological approaches and the huge investments on chemical fertilizers and pesticides known to be environmentally unsafe, productivity still falls short of demand in Nigeria.

In-view of this, national and international bodies have raised a global call to promote maize production through biological approaches, being environmentally friendly and cost-effective. Encouraging the growth of particular group of beneficial soil microorganisms has been reported as a promising alternative to chemical fertilizer (Hayat *et al*., 2010). Reducing fertilizer requirement of major food crop species and biocontrol of plant pathogens are expected to be in-line with this era of sustainable crop production.

Beneficial rhizosphere bacteria, thus, play a pivotal role in crop production by the means of siderophores production, antagonism to soil-borne root pathogens, phosphate solubilization, di-nitrogen fixation and root morphology. These means result in greater root surface area for the transformation, mobilization and solubilization of nutrients from a limited nutrient pool, and subsequently uptake of essential nutrients by plants to realize their full genetic potential (Hayat *et al*., 2010). As the number of beneficial rhizobacteria species increases, it is becoming evident that taxonomical complexity causes ambiguities in the interpretation of individually described

phenotypic markers and the early identification strategies employed over the years have to be revised (Kiska *et al*., 2002).

Traditionally, identification of bacteria has been very time consuming and laborious, except for some pathogenic species for which simple identification methods have been established (Rondon *et al*., 2000). The use of molecular techniques in microbial ecology has made possible the discovery of new microorganisms previously unknown (Macrae, 2000). Fortunately, advances in DNA sequencing technology and high-through-put analytical methods are achieved every year (Ratnasingham and Hebert, 2007). Thus, methods based on DNA sequencing have the potential to be the most generally applicable for rhizobacteria identification; therefore, expanding the taxonomic diversity and utility of tropical rhizobacteria is expected to be explored for biotechnological purposes.

Statement of Problems and Justifications

The absence of a complete culture collection on phyto-beneficial rhizobacteria is one of the most limiting factors in the development of agro-biological based practices on maize growth in Southwestern Nigeria. Studies on phyto-beneficial rhizobacteria have not been undertaken to assess differences among isolates to enhance maize growth with respect to ecological distribution in Southwestern Nigeria. Genetic characterization of isolates using 16S rDNA is necessary to gain insight into the safety and reliability of any phyto-beneficial rhizobacteria with potential for field application and commercialization. Therefore, studies need to be performed to examine the phyto-beneficial effects of rhizobacteria on maize growth, coupled with molecular identification to allow in-depth phylogenetic assessment.

General Objective: This research supports the Millennium Development Goal (MDG) on food security through development of indigenous biofertilizers that sustain and improve maize production in Southwestern Nigeria.

The specific objectives are to determine:

i. bacteria load associated with maize plants in Southwestern Nigeria.
ii. plant growth promoting characteristics of rhizobacteria isolates.
iii. phytobeneficial effects of rhizobacteria on maize growth and nutrients uptake and
iv. molecular characterization of phyto-beneficial rhizobacteria isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspectives of Maize in Nigeria

The name maize is derived from the South American Indian Arawak-Carib word *mahiz*. It is also known as Indian corn or corn in America (Purseglove, 1992). The Portuguese introduced its use to Guinea and the Congo, from where it has become the staple grain crop for much of Sub Saharan Africa. It was introduced into Nigeria probably in the 16th century by the Portuguese (Osagie and Eka, 1998). The first attempt on Agricultural research in Nigeria was made in 1899 (Fakorede *et al.*, 1993). Initially, Agricultural research work in Nigeria was directed at promoting the development of various cash crops including cocoa, oil palm, cotton, groundnut for export purposes. The advent of a very destructive rust disease known as the American rust incited by *Puccinia polestar*, which entered West Africa in 1950, called attention to the importance of maize as food crop. Most of the work done on maize prior to 1950 can be described as agronomic. Research on methods of cultivating maize was to a large extent secondary since the designing of efficient farming system was given priority. Maize was used merely as a test crop for soil fertility to determine the influence of green manure and various sequences of crops in the rotation. Most of this work was done at Moor Plantation in Ibadan, Ogba near Benin City and Umudike near Umuahia (Iken and Amusa, 2004).

The absence of resistance or tolerance in the local maize varieties to the American rust shaped the first approach towards an improvement of the maize crop and introduction of maize materials from all over the world. Subsequently, organized approach towards a systematic study of the crop was established at the Federal Department of Agricultural Research (FDAR) Moor Plantation, Ibadan in 1956. This initial approach was to breed for disease resistance. The screening of local and introduced varieties was the first step towards the recognition of promising maize materials (Iken and Amusa, 2004).

In 1961, some maize varieties were artificially inoculated to test for their quantitative reaction to the Polestar rust under field conditions (Craig, 1962). A total of 137 maize cultivars were screened over a period of three years for resistance to polestar rust, maize blight, Curvularia leaf spot, streak virus and brown leaf spot (Fajemisin, 1978). All the entries exposed to streak had 70% disease incidence. Next to streak, rust appeared to be the most destructive of the diseases, followed by Curvularia leaf spot. The important diseases have changed with time. Streak virus

that was relatively unimportant up to the 1970 is now the most devastating disease of maize nationwide. Downy mildew that was unknown in the early stages of maize improvement came "on stage" in 1975 in some specific zones and is now the most deadly constraint to maize production in the endemic zones. Resistance to both downy mildew and streak had been bred into maize – (DMRSR Varieties) through the effort of research institutions in Nigeria. These varieties produced more than 3t/ha under severe downy mildew pressure during which susceptible varieties gave very low yield of about one tonne per hectare (Fakorede *et al*., 1993).

2.2 Economic importance of maize in Nigeria

Maize is cultivated largely in Nigeria by farmers on subsistence and commercial levels taking about 1.8 million hectares of land, which yields an estimate of 1.5 metric tones (FAO, 2004). Maize is high yielding, easy to process, readily digested, and cheaper than other cereals. Every part of the maize plant has economic value; the grain, leaves, stalk, tassel, and cob can all be used to produce a large variety of food and non-food products (IITA, 2009). Africans consume maize as a starchy base in a wide variety of porridges, pastes, grits, and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled; playing an important role in filling the hunger gap after the dry season. Maize accounts for 30−50% of low-income household expenditures in Eastern and Southern Africa (IITA, 2009).

It is a staple food crop found in the diets of many households in Nigeria. Its vegetative part is used in making silage for ruminants and the maize crop residue is also a useful source of feed for cattle during the dry season. Maize is a good source of energy in poultry feed, and its bye-product is added to pig ration to boost the energy level. Maize supplies raw materials for beverage, soap and pharmaceutical industries (Iken and Amusa, 2004). The economic importance of this crop has been further boosted by the Federal Government who imposed ban on importation of cereals such as rice, maize and wheat since 1986. Local production therefore needs to be stepped up to meet the demand for human consumption, breweries, pharmaceutical companies, baby cereals, livestock feed and other industries (Tijani and Osotimehin, 2007).

2.3 Maize production in Nigeria

Worldwide production of maize is 785 million tons, with the largest producer, the United States, producing 42%. The total production of maize in 2001 was estimated to be about 42 million tons

(FAO, 2002). The largest African producer is Nigeria with nearly 8 million tons, followed by South Africa. Africa imports 28% of the required maize from countries outside the continent. According to 2007 FAO estimates, 158 million hectares of maize are harvested worldwide. Africa harvests 29 million hectares, with Nigeria, the largest producer in SSA, harvesting 3%. Maize improvement work started in the forest zones but yield trials were soon conducted in both forest and savanna locations (Van Eijnatten, 1965). The differences in yield potential of the ecological zones, testing of new maize varieties across the country became an established practice in maize breeding. These trials were called cooperative maize yield trials (Chinwuba, 1962). With time, the name has gone through several changes, including zonal Trials, Uniform maize, variety trials and now, Nationally Coordinated Maize Variety Trials (NCMVT).

Yields in Ibadan (7º22'N) representing the Forest zone and Mokwa (9º19'N) in the Southern Guinea Savanna were much lower than in Savanna (11º11'N) of the Northern Guinea Savanna. A comparison of Forest and Savanna zone yield trials conducted for four years showed that the yield advantage of the Savanna was due primarily to ear number. The savanna zone consistently produced more ears per unit land area. Therefore barrenness was much more pronounced in Forest zone than in Savanna ecologies. Maize plants in the Savanna were taller with higher ear placement, suggesting greater vigor of growth. Number of days to silking was about the same in the two ecologies although the late Ops and the yellow hybrids tended to silk later in the Savanna than in the forest zone. However, percentage moisture content at harvest was consistently lower at Savanna than at forest zone. This implies a shorter grain – filling duration and/or a faster dry-down rate in the Savanna than in forest ecologies. Indeed, the "stay green" character secures frequently in the forest zone, whereas it is almost non-existent in the savanna zones. The hybrid maize project has made an impact in Nigeria. The yield advantages of hybrids appear to be sufficiently large to attract the attention of farmers. Improved high yielding maize variety can express its full genetic potential only when offered optimum management resources (Iken and Amusa, 2004).

2.4 Maize consumption

Worldwide consumption of maize is more than 116 million tons, with Africa consuming 30% and SSA 21%. However, Lesotho has the largest consumption per capita with 174 kg per year. Eastern and Southern Africa uses 85% of its production as food, while Africa as a whole uses

95%, compared to other world regions that use most of its maize as animal feed. Like many other regions, it is consumed as a vegetable although it is a grain crop. The grains are rich in vitamins A, C and E, carbohydrates, and essential minerals, and contain 9% protein. They are also rich in dietary fiber and calories which are a good source of energy. All parts of the crop can be used for food and non-food products (IITA, 2009).

Maize is processed and prepared in various forms depending on the country. Ground maize is prepared into porridge in Eastern and Southern Africa, while maize flour is prepared into porridge in West Africa. Ground maize is also fried or baked in many countries. In all parts of Africa, green (fresh) maize is boiled or roasted on its cob and served as a snack (IITA, 2009). In developing countries, it is mainly used for human consumption and as major source of income to farmers among whom many are resource-poor (Tagne *et al*., 2008). Maize is prepared and consumed in a multitude of ways which vary from region to region or from one ethnic group to the other. For instance, maize grains are prepared by boiling or roasting as paste ('eko'), 'abado', and 'elekute' in Nigeria and 'kenke' in Ghana, or as popcorn which is eaten all over West Africa (Abdulrahaman and Kolawole, 2006).

2.5 Trend in maize production in Nigeria

Until recent years, the bulk of maize grain produced in Nigeria was from the southwest zone. Ogunbodede (1999) reported that western Nigeria generally produced about 50% of Nigeria green maize, the remaining 50% being split between the North and the East. Although large proportion of the green maize is still produce of the south- Western part, there has been a dramatic shift of dry grain production to the savanna, especially the Northern Guinea savanna. This can now be regarded as the maize belt of Nigeria. In this zone, farmers tend to prefer maize cultivation to sorghum. This trend may have been brought about for several reasons including availability of streak resistant varieties for all ecological zones in Nigeria, availability of high-yielding hybrid varieties, increase in maize demand coupled with the federal Government imposed ban on importation of rice, maize and wheat. Local production had to be geared up to meet the demand for direct human consumption, breweries, pharmaceutical companies, baby cereals, livestock feeds and other industrial use (Iken and Amusa, 2004).

2.6 Constrains to maize production

Soil fertility decline is a major problem facing small-scale farming in sub-saharan Africa. Although, inorganic fertilizers are used in the region, the amounts applied are normally insufficient to meet crop demands due to their high costs and uncertain availability. The overall amount of nutrients released from organic amendments for crop uptake depends on the quality, the rate of application, the nutrient release pattern and the environmental conditions (Mugwira and Mukurumbira, 1986; Murwira and Kirchmann, 1993). Unfortunately, for many trials there is lack of crucial information on nutrient content and quality of organic inputs; therefore, it has not been possible to establish quantitative recommendations on the amounts or organic materials needed to obtain similar crop yields as a given amount of fertilizer nitrogen (N). There is an indisputable need to link the quality of the organic material to its fertilizer equivalency value (CAB International, 2002).

The use of mineral fertilizers alone had not solved the problem of crop production in the tropics and adverse side effects of continuous application of acidifying fertilizers like sulphate of ammonia on acid soil are known (Bache and Heathcote, 1969). On the work carried out by Agboola and Obigbesan (1975) it was revealed that a research field which was subjected to 10 years continuous cropping to mainly maize and cowpea with the regular use of only NPK fertilizers resulted into a situation where about 75% of organic matter of the field was lost and the exchangeable K (Potassium) was reduced to 13% of the original value, while P (Phosphorus) content was reduced to one half of its original value. Likewise, over the years, the use of organic materials in farming has been reduced significantly due to chemical fertilizers being rich and ready source of plant nutrients. High population growth rate, rapid urbanization and mechanization have forced the farming community to totally rely on chemical fertilizers. Excessive use of chemical fertilizers, however, created concerns due to energy crises, stagnant yield and soil (Physical, chemical and biological properties) health (Doran and Jones, 1996a).

The continued cultivation and production of maize as a lucrative agribusiness enterprise is however threatened by a number of problems. Maize does not tolerate drought well and the grain can rot during storage in tropical climates. Lack of sunshine can reduce the production potential of the crop (Bartels and Nelson, 1994; IITA, 2009). Various species of stem borers rank as the most devastating maize pests in Sub-Saharan Africa (SSA). Other pests in SSA include ear

borers, armyworms, cutworms, grain moths, beetles, weevils, grain borers, rootworms, and white grubs. The parasitic Striga weed is another maize pest. In fact, weed-related yield losses ranging from 65 to 92% have been recorded in the Nigerian savanna (IITA, 2009). Likewise, most of maize varieties grown in Nigeria are highly susceptible to downy mildew disease (Iken and Amusa, 2004). Other diseases of the crop include maize rust, leaf blight, maize streak, maize mottle / chronic stunt, curvularia leaf spot, stalk and ear rots. In addition, maize parasitic weed, known as striga has been reported to be a serious threat to increased maize production, thereby causing economic losses in Northern Guinea Savanna and some parts of derived southern Guinea. Insect pests, such as stem borers, armyworms, silkworms, grasshoppers, termites and weevils also affect the yield of the crop (Tijani and Osotimehin, 2007). In addition, diseases such as downy mildew, rust, leaf blight, stalk and ear rots, leaf spot, maize streak virus, seedling root rot, stalk rot, and collar rot of seedlings (White, 2000; Andrés-Ares *et al*., 2004) are also among the major factors that limit maize production in Nigeria.

2.7 Application of rhizobacteria as biological control agents

The control of maize diseases is very important as a complementary technology to boost maize production. Various approaches have been used over many decades to control maize diseases which included breeding for resistance and chemical pesticides (Tagne *et al*., 2008). The problems of the chemical pesticides include resistance, pest resurgence, environmental pollution, and risks to human health. Most of the pesticides and inorganic fertilizer's are not environmentally friendly, apart from the fact that health hazards may loom as a result of the consumption of their residues in food. Also, these agrochemicals are expensive and may not be available for farmers use when needed. Likewise, there are legislations against massive use of agrochemicals in crop protection; therefore, a search for natural bioprotectants should be of global interest (Oyekanmi *et al*., 2008). Achieving a sustainable agriculture (Mukerji and Ciancio, 2007) will require the development of new approaches and alternative management and technologies.

Biological control is thus an alternative method that can help in reducing the disease to economical levels (Prabavathy *et al*., 2006; Talubnak and Soytong, 2010) for a more sustainable agriculture (Baniasadi *et al*., 2009). The inconsistent performance of imported biocontrol agents or biofertilizers in the field conditions (Debananda *et al*., 2009) has raised a global call to

develop indigenous strains that will suit local conditions (Khan *et al*., 2002; Oyekanmi *et al*., 2007). That is, why Howel, (2003) suggested that isolation of biocontrol agents should be from the soil locality where it is expected to function as disease control. However, microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for roots against attack by pathogens. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and also during secondary spread on the root (Weller *et al*., 2002). Moreso, the study of root-associated bacteria and their antagonistic potential is important not only for understanding their ecological role in the rhizosphere and the interaction with plants but also for any biotechnological application (Berg *et al*., 2002). Numerous studies have demonstrated the ability of several rhizobacteria to suppress diseases caused by fungal plant pathogens (Emmert and Handelsman, 1999; Kurze *et al*., 2000).

2.8 Application of rhizobacteria towards crop improvement

The importance of specific microorganisms that are part of the microbial biodiversity of a soil-ecosystem and how to use them for development of innovative biological control technologies has been only superficially researched in Africa (Sikora *et al*., 2003). Since the beginning of second millennium, there has been serious investigation on soil microorganisms as potential biocontrol agents of plant pathogens in Africa (Adekunle *et al*., 2001; Bacon *et al*., 2001; Cardwell and Cotty, 2002). At present, the use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system. In this regard, the use of Plant Growth Promoting Rhizobacteria (PGPR) has found a potential role in developing sustainable systems in crop production (Sturz *et al*., 2000; Shoebitz *et al*., 2009). A variety of symbiotic (*Rhizobium* sp.) and non-symbiotic bacteria (*Azotobacter, Azospirillum, Bacillus*, and *Klebsiella* sp., etc.) are now being used worldwide with the aim of enhancing plant productivity (Burd *et al*., 2000; Cocking, 2003). PGPR have the potential to contribute in the development of sustainable agricultural systems (Hayat *et al*., 2010).

Generally, PGPR function in three different ways (Glick, 1995; 2003) synthesizing particular compounds for the plants (Dobbelaere *et al*., 2003; Zahir *et al*., 2004), facilitating the uptake of certain nutrients from the soil (Lucas *et al*., 2004a, b; Çakmakçi *et al*., 2006), and lessening or

preventing the plants from diseases (Raj *et al*., 2003; Saravanakumar *et al*., 2008). The mechanisms of PGPR-mediated enhancement of plant growth and yield of many crops are not yet fully understood (Dey *et al*., 2004). However, the possible explanation include (1) the ability to produce a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in the root of developing plants thereby increasing the root length and growth (Li *et al*., 2000; Penrose and Glick, 2001); (2) the ability to produce hormones like auxin, i.e indole – 3- acetic acid (IAA) (Patten and Glick, 2002), abscisic acid (ABA) (Dangar and Basu 1987; Dobbelaere *et al*., 2003), gibberellic acid (GA) and cytokinins (Dey *et al*., 2004); (3) a symbiotic nitrogen fixation (Kennedy *et al*., 1997, 2004); (4) antagonism against phytophatogenic bacteria by producing siderophores, ß-1, 3-glucanase, chitinases, antibiotic, fluorescent pigment and cyanide (Cattelan *et al*., 1999; Glick and Pasternak, 2003); (5) solubilization and mineralization of nutrients, particularly mineral phosphates (Richardson, 2001; Banerjee and Yasmin, 2002); (6) enhanced resistance to drought (Alvarez *et al*., 1996), salinity, waterlogging (Saleem *et al*., 2007) and oxidative stress (Stajner *et al*., 1995, 1997); and (7) production of water-soluble B group vitamins niacin, pantothenic acid, thiamine, riboflavine and biotin (Sierra *et al*., 1999; Revillas *et al*., 2000). The application of PGPR has also been extended to remediate contaminated soils in association with plants (Zhuang *et al*., 2007). Thus, it is an important need to enhance the efficiency of meager amounts of external inputs by employing the best combinations of beneficial bacteria in sustainable agriculture production systems. The intensive research on plant growth promoting bacteria (PGPB) is underway worldwide for developing biofertilizers and biocontrol agents (BCA's) as bio-inoculant components in organic agriculture and better alternatives to agrochemicals as chemical fertilizer harm the environment and human health besides the high cost of purchasing chemical fertilizer (Debananda *et al*., 2009). The further elucidation of different mechanisms involved will help to make these bacteria a valuable partner in future agriculture as it has been used to enhance growth of Rice (Baldani *et al*., 2000; Engelhard *et al*., 2000), Maize (Estrada *et al*., 2005), Sugarcane (Suman *et al*., 2005, 2007), Wheat (Roesti *et al*., 2006), Blackgram (Selvakumar *et al*., 2009), Mungbean (Nazmun Ara *et al*., 2009) and many more.

Despite the fact that, rhizobacteria have been recognized has potential bioprotectant or biofertlizer (Talubnak and Soytong, 2010) to enhance organic farming yet remain largely untapped to improve maize production in Nigeria probably due to paucity of knowledge in the

genetic and functional relationship among isolates which could be of help to rapid selection of useful isolates before field application (Hill *et al*., 2000). As beneficial rhizobacteria are being developed to biofertilizers and to replace chemical pesticides, it becomes increasingly very important to gain insight into phyto-beneficial effects and genetic relationship among rhizobacteria isolates which could be of help to better management and improvement of maize in Nigeria.

2.9 Molecular characterization of beneficial rhizobacteria

Analysis of genotypic and phenotypic characteristics of indigenous rhizobacteria has been reported as a tool to clarify the mechanisms involved (Di Cello *et al*., 1997) in elucidating the genetic basis of beneficial rhizobacteria (Bloemberg and Lugtenberg, 2001). Also, the use of Polymerase Chain Reaction (PCR) and its utility in providing an accurate characterization of the (rhizo) bacteria community, speed, sensitivity and relatively low cost far out-weigh any disadvantages, while providing tremendous benefit, above and beyond traditional methods (Rondon *et al*., 2000; Ranjard *et al*., 2001; Zwolinski, 2007). Presently, there are too few or no data on molecular characterization of indigenous phytobeneficial rhizobacteria isolates, likewise, significance of phylogenetic studies based on 16SrDNA sequences is increasing in the systematics of bacteria (Yokota, 1997; Smith, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Geographical focus: Southwestern Nigeria

3.2 Ecological zones: The ecological zones in southwestern Nigeria are: Guinea savannah, Derived Savannah, Lowland rainforest, Freshwater swampy forest, Mangrove forest / Coastal vegetation.

3.3 Commodity base: Maize

3.4 Field survey, study area and selection of site: Four study areas of about 20km were randomly selected and surveyed in each ecological zone in southwestern Nigeria. In each study area, five sites of about 100m apart were randomly selected for sampling (Figure 3.1).

3.4.1 Collection of soil samples: Rhizosphere soils were collected from soils adherent to maize roots to a depth between 5 and 15cm, while their control bulk soils were sampled 2m away from the maize plants. The core samples were mixed together to form a composite in each study area, while the composites from each study area were pooled together to form a composite for each ecological zone.

3.4.2 Sterilization of glassware and other equipment: All glassware (Petri dishes (Pyrex), pipettes, beakers, test tubes and conical flasks) were washed with teepol (liquid detergent) rinsed with several changes of tap water and air-dried. Petri dishes and pipettes were arranged inside canisters. These canisters and other glassware were arranged inside an oven (Model Gallenkamp Hotbox Oven, Gallenkamp, UK), and ample space was left between items to allow for air circulation and to avoid breakage. The oven was heated at 160^{o}C for 1hour and the sterilized glasswares were used within 2 days.

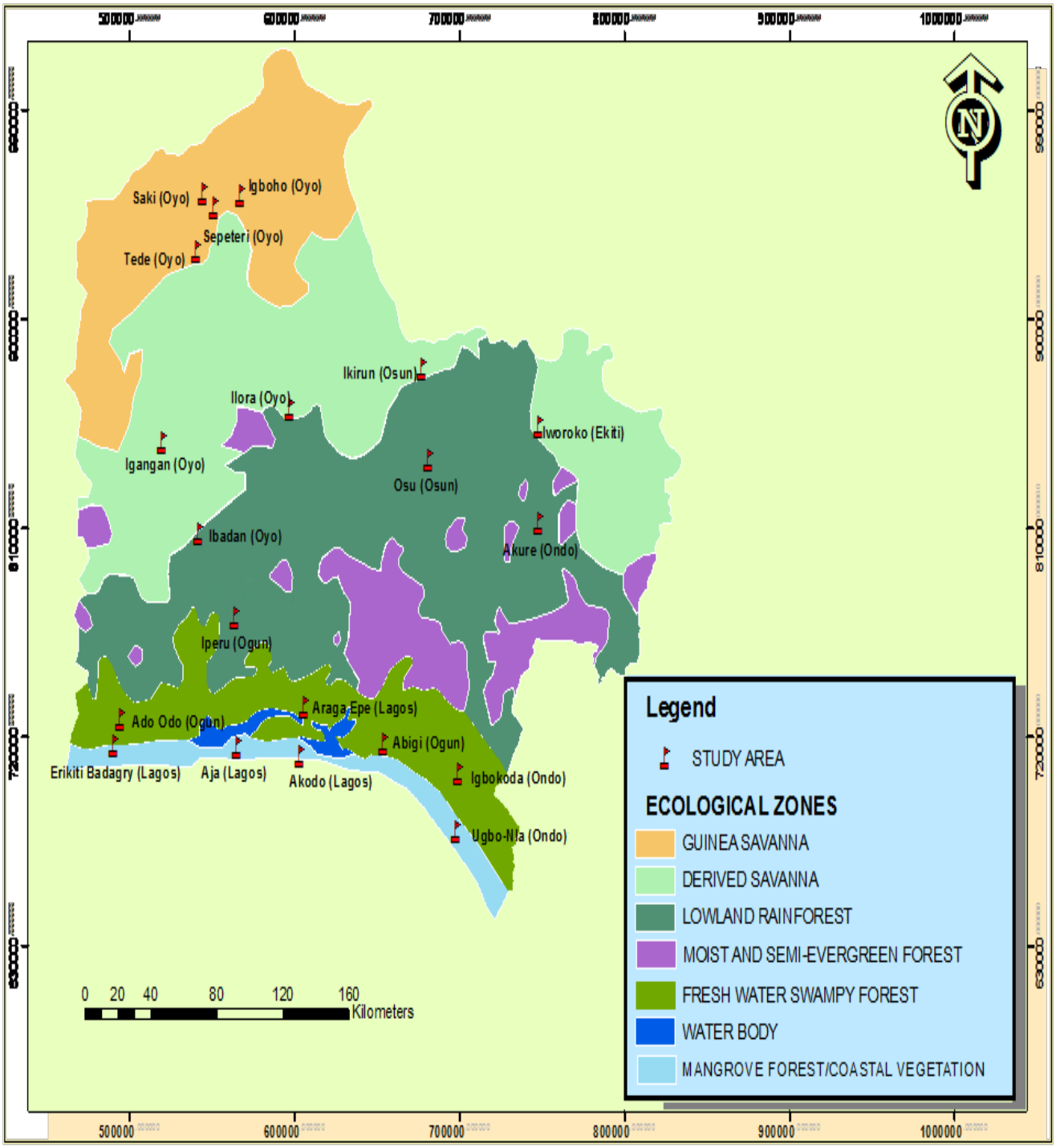


Figure 3.1: Study areas in ecological zones of Southwestern Nigeria

3.4.3 Sterilization of Laboratory utensils: Forceps, lids of flasks, the sides of Petri dishes, microscope-slides, cover slips and inoculating loops were sterilized by holding each of these materials at a 45^{o}C angle in the upper portion of a flame from a Bunsen burner.

3.4.4 Laboratory experiments: Innoculating loop was sterilized by heating to 'Red hot', while glass objects, cork borers and inoculating needles were dipped inside 80% ethanol and passed through the Bunsen flame several times.

3.4.5 Sterilization of Laminar flow work station and laboratory benches: The Laminar flow and laboratory benches were cleaned with mixture of teepol and 5% sodium hypochlorite. Thereafter, the laminar flow was surface sterilized with a 70% ethanol. Fan attached to the hood was switched on to blow a steady stream of microbe-free air across the working surface for about 15 minutes before the commencement of the laboratory analysis.

3.4.6 Preparation and sterilization of culture media: Nutrient agar (for the bacteria) and Potato Dextrose Agar (for the pathogenic fungi) both media were prepared according to manufactures' specifications and instructions and autoclaved at 121^{o}C for 15minutes at 15-psi pressure. Each medium was prepared in 1 litre of distilled water in 2-litre conical flasks. The media was pre-heated in a water bath (Model HAAKE SWB 20) at 100^{o} C for 25 minutes to dissolve the agar completely. The conical flasks containing agar suspensions were cotton-plugged with non-adsorbent cotton wool wrapped with aluminium foil. Distilled water was poured into the chamber of a Market Forge Sterilmatic, Everett, autoclave and all the air was removed before closing the exhaust valve. The media were autoclaved and allowed to cool to 45^{o}C inside the laminar flow hood (Model EACI ENVIRCO) and approximately 12 – 15ml quantity was poured into already sterilized petri dishes. The poured plates were left at room temperature inside laminar flow hood to solidify. Before plating, plates were dried, open and inverted at 50^{o}C for 30 minutes to remove condensed water on the agar surface.

3.4.7 Isolation of bacteria: Serial dilution- pour plate technique was used to carry out the isolation. Inoculated petri plates were sealed with parafilm and incubated at temperature of 25^{o}C for 24 hours.

Serial dilution-pour plate technique: From each soil sample, ten (10g) grams was weighed into sterile 250ml Erlenmeyer flask containing 90ml of sterile distilled water, the flask was shaken

well on wrist action shaker to homogenize the suspension (this is the 10^{-1} dilution). Thereafter, 1ml aliquot from 10^{-1} dilution was measured into another test tubes containing 9ml of sterile distilled water to obtain 10^{-2} dilution. Further dilutions were carried out till a dilution level of 10^{-6} (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}). Starting from the highest dilution (10^{-6}) and subsequently from 10^{-0} and 10^{-3}, 0.5ml of the suspension was inoculated into already sterilized petri dishes and 12 - 15 ml of sterilized nutrient agar was poured aseptically into the already inoculated petri dishes, swirled and allowed to solidify in the laminar flow cabinet. The petri plates were sealed with the parafilm, inverted and incubated at 25 ± 2^{o}C, thereafter, the petri plates were observed after 24 hours. The number of colonies per petri dish was recorded accordingly at different dilution factors.

3.4.8 Enumeration of bacteria: Colony of bacteria per gram of soil using the following formular was calculated for 10^{6}.

CFU / 1gram of soil = Number of colonies / plate x dilution factor.

3.4.9 Purification of bacteria isolates: Isolates which are different in morphological appearance were selected and purified by streaking until pure cultures were obtained.

3.4.9.1 Maintenance of cultures: All pure culture of bacteria isolates were inoculated and maintained on slants, stored at 4^{o}C in a refrigerator and were subcultured monthly. Also, in glycerol based media and stored at -20^{o}C.

3.5 Geographical factors

- Data on the latitude, longitude and altitude / elevation of the sample areas were collected using 12 channels geographical positioning system (GPS) model (etrex, Garmin Corporation Multi-lingual).
- Data on the average temperature, rainfall and precipitation during the experimental period were obtained from Nigeria Meteorological Agency.

3.6 Physico-Chemical analysis of collected soil samples

Soil analyses for the screenhouse experiment were carried out in the Analytical Service Laboratory (ASLAB) of the International Institutes of Tropical Agriculture (IITA), Ibadan, Oyo

State, Nigeria. The soil samples collected were air dried and sieved through 2 mm and 0.5 mm sieve for physical and chemical analyses.

3.6.1 Chemical analysis of collected soil samples

Soil total nitrogen (N) was determined by the macro-Kjedahl method (Bremmer and Mulvaney, 1982) and colorimetric determination on Technicon Autoanalyser (1971) and organic carbon by chromic acid digestion (Heanes, 1984). Phosphorus and exchangeable cations (Ca, Mg, K and Na) measurements were done by Mehlich 3 extraction (Mehlich, 1984) and phosphorus was determined colometrically using the Technicon AAII Auto-analyser, while the cations (Zn, Cu, Mn and Fe) were determined using atomic absorption spectrophotometer (Model Buck 200A).

3.6.2 Physical (Mechanical) analysis of collected soil samples

3.6.2.1 ***Determination of particle size of the soil samples:*** The Bouyoucos hydrometer method of Day (1965) as described by Sheldrick and Wang (1993) was employed in the determination of particle size of the soil samples. Triplicate subsamples from each location were mixed together to get the composite soil sample for each location. Fifty-one grams of each composite sample was weighed out and transferred to a big container of a high speed shaker so that 25ml of freshly prepared 5% sodium hexametaphosphate (calgon) and 400ml tap water were added to the sample in the container. The container was shaken for 2 hours in a mechanical shaker for particle size separation. Samples were then transferred into a 1L measuring cylinder and made to mark by adding tap water before stirring with a paddle for 1 min. The soil hydrometer (Model: ASTM-E100 152H-62, Serial number: 0252, G.H. ZEAL, UK) was introduced into the cylinder and allowed for 20 seconds before taking the first reading (B) after 4 minutes 48 seconds (silt + clay). The second reading (A) was taken 5 hours later for clay. The formula below was used to deduce the sand, silt and clay percentages. Soil textural determination was made by plotting clay, sand and silt percentages onto the textural triangle for soil classification of USDA (1962).

Clay (%) = $[(A\ (gL^{-1}) \times 100) / 50g] - 1$

Silt + Clay (%) = $[(B\ (gL^{-1}) \times 100) / 50g] - 1$

Where 1= calgon correction Silt (%) = [(Silt + Clay) – Clay] %

Total sand (%) = [100 – (Silt + Clay)] %

3.6.2.2 ***Determination of soil moisture content:*** Fifty grams fresh soil samples were measured separately in clean moisture cans; weight of the moisture can and the soil was taken together before and after oven drying to constant weight at 105ºC ± 3ºC for 24 hours. Difference of moisture content of the soil was recorded and calculated. The experiment was done in replicates.

3.6.2.3 ***Determination of soil pH:*** Twenty five **(**25) grams (field moist soil) were taken into a clean dry 150 ml beaker and 50 ml distilled water was added. The contents were thoroughly stirred with magnetic stirrer using magnetic base stirring machine (Nuova II, stirrer {thermolyne}) at 4 rpm. The pH of the suspension was measured with pH meter. pHo p(R))-pH Tester CE from Hanna Instruments, Italy. The experiment was done in replicates.

3.7 Evaluation of plant growth promoting characteristics of bacteria isolates

3.7.1 ***Antagonistic bioassay:*** Antagonistic bioassay was carried out to screen for antagonistic bacteria isolates.

3.7.2 Source of *Fusarium verticillioides* for primary screening: Already confirmed and identified phytopathogenic and highly virulence *Fusarium verticillioides* was collected from Dr. Killani of Soil Microbiology unit of International Institutes of Tropical Agriculture (IITA).

3.7.3 In-vitro antagonistic bioassay: Primary screening: An in-vitro bioassay was conducted to evaluate the antagonistic potentials of bacteria isolates against phytopathogenic *Fusarium verticillioides*. Centre streak method (Kim *et al*., 1998b; Hassanein *et al*., 2002) was used. Nutrients Yeast Broth Agar (NYBA) plates were prepared and inoculated with each of the bacteria isolates separately and in replicates by a single streak inoculum in the centre of the petri dish. After 24 hours of incubation, agar plug of fungus mycelium was placed near the edge of each bacteria inoculated plates in replicates. The plates were then incubated at 28ºC for 7 days. Experiment was repeated to reconfirm (post-primary screening) the antagonistic potentials of the bacteria isolates. Results were scored after 7 days for antagonist-pathogen interaction. Presence (+) or absence (-) of antagonisim by each of the bacteria isolates were observed.

3.7.4 Phosphate solubilization: The potentials of bacteria isolates to solubilize phosphate were carried out as modified from the method of Sharma *et al*. (2007). The phosphate solubilization ability of the bacteria was tested on compounded modified agar. Five (5) grams of $CaHPO_4$ was used as the source of phosphate and co – compounded with 2.5 g of glucose, 1g of $MgSO_4$ $7H_20$,

20g of agar agar and distilled water (to a litre), pH 6.8. The media bottle containing the agar suspensions was well corked and covered, autoclaved at 121^{o}C, for 15 minutes at 15-psi pressure. The autoclaved medium was then allowed to cool to 45^{o}C inside the laminar flow hood (Model EACI ENVIRCO) and approximately 12 – 15ml quantity was poured in already sterilized petri – dishes. The poured plates were left at room temperature inside laminar flow hood to solidify. Each isolate was spot inoculated on the modified medium for detection of their phosphate solubilizing ability and incubated at temperature of 37^{o}C for 48 hours. Halo surrounding the colonies were measured and the solubilising efficiency (SE) was calculated by the following formular;

$$SE = \frac{\text{Solubilization diameter} \times 100}{\text{Growth diameter}}$$

3.7.5 Indole-3- Acetic Acid (IAA) production: The potentials of bacteria isolates to produce indole – 3 – acetic acid was carried out according to the method of Khakipour *et al*. (2008). Ten (10) millilitre of 24 hours old culture of each of the bacteria in broth was transferred to 10ml tips and centrifuged in a refrigerator centrifuge for 10 minutes at 15000 rpm. Then, 2 ml of the supernatant was mixed with 4ml of Salkowsky indicator (containing 150 ml of HCL, 250 ml of purified water and 7.5 ml of 0.5 molar $FeCl_2$) and kept in the darkroom. After 20 minutes of the mixture in the dark room, colour (pink) variation was observed among the bacteria isolates tested. Spectrophotometer at 540 nm wavelength was used to measure the light absorption. The amount of IAA produced was calculated with the comparison of the light absorption of standard curve to determine auxin production of isolates in mg / litre indole – acetic density. Three replicates were used for each treatment.

3.7.6 Chitinase enzyme activity: The bacteria isolates were screened to determine their potentials to produce chitinase enzyme according to the method of EL – Mehalawy (2004). Colloidal Chitin Agar (CCA) was prepared according to manufacturer's specification and instructions. Each of the bacteria isolates were inoculated on CCA and incubated at 28^{o}C in the dark until zones of chitin clearing were seen around colonies (mm) and that was used to indicate the chitinase activity of each isolate. Percentage of chitinase activity of each isolate was calculated with the formular;

$$\text{Percentage of chitinase activity (\%CA)} = \frac{\text{Clear zone diameter x 100}}{\text{Growth diameter}}$$

3.7.7 Source of seed varieties: The most commonly grown maize variety in southwestern Nigeria- SUWAN-1-Y was used for laboratory and screen house experiment.

3.7.8 Sterilisation of seed samples: The maize seeds were surfaced sterilized with 0.5% NaOCl for 2 minutes followed by a 30 seconds dip in 70% ethanol and two rinses in distilled water, and allowed to air dried under laminar air flow as described by Dietmar *et al*. (2008) and Zinniel *et al*. (2002).

3.7.9 Seed inoculation: Seeds were inoculated by immersion into suspension of each of the bacteria in separate beakers having inoculum size of $1x\ 10^6$ for 30 minutes and allowed drying on already sterilized paper towel in a laminar flow cabinet for 1-2 hour(s) and later used for seed germination bioassay.

3.7.9.1 Seed germination bioassay

The effect of each bacteria isolates on maize seed germination was carried out in the laboratory using blotter techniques method according to ISTA (1999). Ten (10) inoculated seeds were placed at approximate equidistant position to each other into 9 cm diameter petri dishes already underlay with sterilized moistened filter paper and incubated for 7 days at temperature of 25^{o}C. The germinated seeds were counted at day 7.

Percentage seed viability was determined using the formular: Sv = n/Nx100. Where Sv is % seed viability, n is the number of seeds that germinated and N is the total number of seeds plated.

The radicle and the plumule length were measured after day 7 with thread and the precision was determined on ruler. This experiment was done in replicates and the result was compared with the control (water).

3.8 Soil Sterilization

Topsoil was collected from the farm area near senior staff quarters of Institute of Agricultural Research and Training (IAR&T) and was sieved through 2-mm mesh to remove plant roots and soil debris. Ten (10kg) kilogram of the subsoil was packed inside autoclave bags and sterilized in

the autoclave (Model Market Forge Sterilmatic, Everett) at 121^{o}C for 1 hour at 1.99 bar or 15-psi pressure.

3.9 Pathogenicity test

Prior to antagonist-pathogen interaction study, pathogenic effect of *Fusarium verticilliodes* was evaluated on maize plant according to Koch's postulate (Koch, 1891; Fredricks and Relman, 1996).

3.9.1 Antagonist-pathogen interaction (Biocontrol activity of isolated bacteria)

Baniasadi *et al*. (2009) method was modified to determine the antagonist-pathogen interaction effect on maize seedling growth. Experiment was set up in the screenhouse with plastic pot each containing 2.5kg of sterilized soil. A conidial suspension of phytopathogenic *Fusarium verticilliodes* was prepared from cultures grown on petri-dishes containing 15ml of PDA. Spore suspension of phytopathogenic *Fusarium verticilliodes* was prepared and adjusted to 1 x 10^6 spores / ml. Direct soil inoculation was done with 50ml of sterile distilled water and 4ml of *Fusarium verticilliodes* (1 x 10^6 spores / ml) inoculum (Ros *et al*., 2005) and covered with black polythene bag. After 48 hours, 10 maize seeds SUWAN-1-Y already inoculated (treated) with each bacterium isolate were planted at depth of 2 cm into the treated soil. Watering and weeding were done throughout the experimental period. The untreated maize seeds were the negative control (maize alone) while the phytopathogenic *Fusarium verticilliodes* treated maize seeds were the positive control (maize + fungal pathogen). Pots were replicated three times in complete randomized design. Antagonist-pathogen interaction effect on maize seed germination was determined after day 7. Percentage seed germination was determined using the formular: %G = n/Nx100. Where %G is the percentage seed germination, n is the number of seeds germinated and N is the total number of seeds planted. Occurrence of seedling disease (disease expression) was determined at day 14 and 21. The maize plant height (cm), stem girth (cm), number of leaves and leaf area (cm^2) were measured. Rhizosoil samples as adopted by Louw and Webley (1959) were taken at day of harvest (six weeks of planting). The total bacteria count was estimated on NA using serial dilution-pour plate method as described in section 3.4.7.

3.9.2 Effects of bacteria isolates on maize growth and nutrient uptake

Bacteria isolates that exhibited plant growth promoting characteristics were evaluated in a conventional screenhouse on maize according to the method of Jensen and Fenical (2002) in both sterilized and unsterilized soil. The experiment was set up to compare the potentials of each of the bacteria (treatment) to enhance maize growth and nutrient uptake in maize to that of NPK 15-15-15. The soil and maize seed sterilization were carried out in section 3.7.8. Pre-cropping analysis of Nitrogen (N), Phosphorus (P) and Potassium (K) content in both sterilized and unsterilized soil were carried out. Water holding capacity of soil was determined in the laboratory to know the quantity of water to be applied during the active growing period. Fifty (50) millilitre of sterile distilled water and 6ml of bacteria were added into 2.5 kg of soil (sterilized and unsterilized) in each pot to reach the soil level around $10^6 CFUg^{-1}$ as modified from the method of Ros *et al*. (2005). Pots were replicated three times and arranged in complete randomized design in the screenhouse. Four seeds of SUWAN-1-Y were planted in the 2.5 kg soil (sterilized and unsterilized) at a depth of approximately 2 cm below the soil surface. Watering and weeding were done throughout the experimental period. The seeds were later thinned to two plants / pot after two weeks. Maize seeds planted with NPK chemical fertilizer were the positive control (maize + NPK) while maize seeds planted without any treatment (maize alone) were the negative control. They were grown for six weeks and the following growth parameters were taken at two weeks interval; plant height (cm), stem girth (cm), number of leaves and leaf area (cm^2). At six weeks after planting (6WAP). Maize shoots were harvested, oven dried at 70^oC to a constant weight and recorded as dry matter yield, dried, milled using Willey E.D. 5 milling equipment and analyzed for N, P and K. Rhizosoil samples as adopted by Louw and Webley (1959) were taken at day of harvest. The total bacteria count was estimated on NA using serial dilution-pour plate method described in section 3.4.7.

3.9.3 Plant nutrient analysis

The maize plant analysis was carried out in the Analytical Service Laboratory (ASLAB) of the IITA, Ibadan, Oyo State, Nigeria. Maize plants samples were collected per treatment after the experiment analyzed for N, P and K and were compared to that of controls. The sample leaves were oven-dried at 70^oC to a constant weight. The dried samples were then grounded to pass through a 2mm mesh. Two (2 g) grams of dried and ground leaf samples were digested in hot

sulphuric acid solution using selenium black powder as catalyst (Novozamky *et al*., 1983). The digested solutions were then read colometrically in an auto-analyzer for the simultaneous determination of N and P as follows: The auto-analyzer component was switch on for 30 minutes before the reading were taken. K was determined by flame photometry (Okalebo *et al*., 1993).

3.9.4 Physico-Chemical analyses of post-experimental rhizosoil

The soil pH was determined as described in section 3.6.2.3. The chemical analysis of the post experimental rhizosoil was determined only for N, P and K so as to know the performance of the beneficial bacteria isolates in comparison to NPK chemical fertilizer. The total N was determined using macro-Kjedahl method (Bremmer and Mulvaney, 1982) while P and K were determined by the method of Mehlich (1984).

3.9.5 Preliminary Identification of phytobeneficial rhizobacteria isolates.

The biochemical tests, viz, gram staining, catalase test, starch hydrolysis, casein hydrolysis, growth in 4% NaCl, gelatin hydrolysis and sugar fermentation following the standard protocol (Harrigan *et al*., 1996) were performed.

3.9.6 Molecular characterization of phytobeneficial rhizobacteria isolates

3.9.6.1 ***Extraction of DNA*****:** Bacteria culture (5 ml) was grown in replicates in a NA broth shake culture, 1.5ml of the culture was centrifuged at 1000 rpm for 5 minutes. The obtained pellet was re-suspended and washed with 400µl of phosphate buffer solution (PBS). Re-suspended pellets were centrifuged at 14000 rpm for 5 minutes and the supernatant was decanted. The process was repeated and the pellets that remained were re-suspended with 200µl of PBS for DNA extraction. The DNA extraction from the beneficial bacteria isolates was performed using DNA extraction kit (ZR Fungal / Bacteria DNA MiniPrep ^{TM} – Catalog Number D6005) according to manufacturer's (Zymo Research Corporation) instructions.

3.9.6.2 ***PCR amplification and gel electrophoresis*****:** The 16SrDNA gene was amplified using universal primers for bacteria (Blackwood *et al*., 2005). The PCR was carried out in 25µl volumes containing 1.5mM $MgCl_2$, 0.125U/µl Taq polymerase, 2.5mM of each dNTP, 0.25µM of forward (8f) primer (5'-AGAGTTTGATCCTGGCTCAG-3'), 0.25µM of reverse (1392R)

primer (5'-ACGGGCGGTGTGTAC-3') and 0.5µl template DNA. The PCR was performed under the following conditions; 10 minutes at 95^{o}C, followed by 30 cycles of 45 seconds at 95 ^{o}C, 30 seconds at 54 ^{o}C, 90 seconds at 72 ^{o}C and then final extension at 72^{o}C for 5 minutes, while the holding temperature was 4 ^{o}C. The PCR – amplified samples were loaded on a 1.5% agarose gel with loading dye and run at 220V for 45 minutes at room temperature with a 1X TAE (Tris – Acetate - EDTA) buffer. The gels were stained with ethidium bromide for 15 minutes and visualized with a UV transilluminator.

3.9.6.3 ***Purification of DNA, quantification of DNA and nucleotide sequence determination*****:** The PCR amplified reaction product was purified using the "UltraClean® PCR Clean – Up Kit with Catalog Number #: 12500-100 according to manufacturer's (MO BIO Laboratories, Incorporation) instructions. DNA quantification followed PCR purification using PicoGreen DNA assay (to quantify the amount of double stranded DNA present in a sample). Prior to PicoGreen assay, DNA standards using Lambda DNA stock at different range of concentrations and the blank were prepared. Exactly 100µl of dilute PicoGreen per sample to be assayed was made (including the standards). PicoGreen stock was diluted 1: 200 with 1XTE buffer (1µl PicoGreen to 199µl buffer). Using a black microplate, 2µl of samples / standard was pipetted into each well. Exactly 98 µl of diluted PicoGreen was added to each well, and allowed to stay for 5 minutes for the dye and DNA to bind. The DNA was quantified using fluorescence microplate reader (BioTek Plate Reader by BioTek Instruments, Incorporation, Highland Park, Winnooski, Vermont, U. S. A), and the R^2 value from the standard curve was recorded. The already purified and quantified DNA was sequenced at Plant – Microbe genomic facility, Ohio State University. Likewise, nucleotide sequences determinations were re-confirmed at Advanced Genetic Technologies Center (AGTC), University of Kentucky.

3.9.6.4 ***Phylogenetic analysis*****:** Sequences were taxonomically classified using Naïve Bayesian Classifier implemented by the Ribosomal Database Project (Wang *et al*., 2010). The taxonomic placement was based on 80% confidence level. Sequences were edited using Bioedit version 7.0.5 (Brown, 1999) and aligned using the CLUSTAL W programme (Thompson and Gibson, 1997). Phylogenetic history was constructed using software MEGA 5 (Tamura *et al*., 2011). Evolutionary history was inferred using the Neighbor – Joining method (Saitou and Nei, 1987). The phylogenetic tree was drawn to scale, with branch lengths in the same units as those of the

evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbor joining data set.

3.9.7 Combined effects of phyto-beneficial rhizobacteria on maize growth and nutrient uptake

This experiment was set up to compare and checkmate combined effects of beneficial bacteria to that of NPK chemical fertilizer on maize growth and nutrient uptake. Based on the above data, the best known beneficial bacteria from each ecological zone (GS= *Myroides odoratus*, DS= *Bacillus niacin*, LR= *Enterobacter pyrinus*, FW= *Lysinibacillus xylanilyticus*, MF= *Stenotrophomonas nitritireducens*) were combined together and evaluated as biofertilizer in comparism to NPK chemical fertilizer on maize growth and nutrient uptake. Plastic pots containing 2.5kg of sterilized soil were replicated three times and arranged in complete randomized design. Prior to inoculation, equal volume (10ml) of each of the bacteria cell suspension were aseptically combined together and homogenized. Exactly 50ml of sterile distilled water and 6ml of the combined bacteria inoculum were added into 2.5kg of soil to each pot to reach the soil level around $10^6 CFUg^{-1}$, a modified method of Ros *et al*. (2005). Four seeds of SUWAN-1-Y were planted in the 2.5kg sterilized soil at a depth of approximately 2cm below the soil surface. The treatment was as follows; T1= Maize + Combined beneficial Bacteria (CPB), T2=Maize + NPK, and T3=Maize + water (maize alone, that is, without any treatment). Watering and weeding were done throughout the experimental period. The seeds were later thinned to two plants / pot after two weeks. The planted maize was grown for six weeks and the following growth parameters were taken at two weeks interval; plant height (cm), stem girth (cm), number of leaves and leaf area (cm^2). At six weeks after planting (6WAP) maize shoots were harvested, oven dried at 70^oC to a constant weight and recorded as dry matter yield. This was later dried, milled using Willey E.D. 5 milling equipment and analyzed for NPK. Rhizosoil samples as adopted by Louw and Webley (1959) were taken on the day of harvest. The total bacteria count was estimated on NA using serial dilution-pour plate method described in section 3.4.7.

3.9.8 Data collection and statistical Measures

Data analysis was performed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) 2009 software version 9.2 (SAS Institute, Inc, Cary, NC). The means of the data obtained from the soil pH, moisture content (%) and bacteria load at different dilution factor were analyzed and means were separated and compared with standard error using Tukey – Kramer HSD test at $\alpha = 0.05$.

To determine the plant growth promoting characteristics of the isolates in the laboratory, data obtained from phosphate solubilization efficiency (%), chitinase activity (%), IAA, maize seed germination in the laboratory, radicle length, plumule length, maize seed germination in the screenhouse, disease expression, plant height, stem girth, leaf number, leaf area and bacteria load were analyzed by ANOVA, means were separated using Student – Newman – Keuls (SNK) test at $\alpha = 0.05$.

Pearson's correlation coefficients for pathological and agronomic data obtained on screening procedure to determine the biocontrol activity of isolates were computed using SAS descriptive correlation to show the relationship between disease expression and plant morphogenesis (plant height, stem girth, leaf number, leaf area, dry matter yield, soil pH and bacteria load) as influenced by the antagonists (bacteria isolates).

The means of parameters obtained from beneficial effects and combined effects of beneficial bacteria isolates on maize growth and nutrients (nitrogen, phosphorus and potassium) uptake were analyzed and separated using Tukey – Kramer HSD test at $\alpha = 0.05$ with respect to weeks of planting and type of soil used. Likewise, means of measurable variables were correlated using SAS descriptive correlation while regression of variables were analysed using Microsoft excel 2010.

CHAPTER FOUR

RESULTS

4.1 Field survey information

The field survey information (Figure 3.1 and Table 4.1) revealed five (5) ecological zones, namely; Guinea Savannah (GS), Derived Savannah (DS), Lowland Rainforest (LR), Fresh Water swampy forest (FW) and Mangrove Forest (MF). The latitude, longitude and elevation for each of the study area in consonance with their ecological zone were presented in table 4.1. The latitude ranged between 6.3645 (lowest) in Igbokoda to 8.6293 (highest) in Saki while the longitude ranged from 2.9179 (lowest) in Badagry to 5.2514 (highest) in Akure. Also, 505m was recorded for Saki in GS ecological zone as the highest elevation while the lowest elevation, -3m (below sea level) was recorded for Akodo in MF. During the period of samples collection in the study areas, 89.5% of the farmers planted Suwan-1-Y (SW1), followed by 15.8% TZL-Composite 4-C2 (TZL) while 10.5% planted OBASUPER 2 (Table 4.1 and Figure 4.1).

The use of plant manure predominated (Table 4.1) all the study areas in all the ecological zones while that of animal manure was observed at Ilora (Derived savannah) and Badagry (Mangrove forest). Likewise, the use of NPK chemical fertilizer was observed as irregular among the farmers in the study areas (Table 4.1) but was consistently used by farmers in Akure study area (Lowland rainforest), Ado-Odo (Freshwater swampy forest), Epe (Freshwater swampy forest) and Badagry (Mangrove forest).

4.2 Physical properties of collected soil samples

The soil pH of Tede (6.53), Igangan (6.40), Sepeteri (6.37), Iworoko (6.37) were not significantly ($P < 0.05$) different from each other but were observed to be significantly ($P < 0.05$) different from other study areas. Similarly, the soil pH of Ikirun (6.20), Iperu (5.97), Igboho

Table 4.1: Field Survey Information (Ecological zones in Southwestern Nigeria)

Ecological zones	State	Study area	Maize varieties	NPK Fertilizer Before	NPK Fertilizer Now	Plant Manure	Animal Manure	Latitude	Longitude	Elevation
Guinea	Oyo	Saki	SW 1	-/+	-	+	-	8.6293	3.4104	505M
Savannah	Oyo	Tede	SW 1	-/+	-	+	-	8.4024	3.3725	357M
(GS)	Oyo	Igboho	SW 1	-/+	-	+	-	8.6218	3.6155	359M
	Oyo	Sepeteri	SW 1	-/+	-	+	-	8.5731	3.4765	392M
Derived	Oyo	Igangan	SW 1, TC4	-/+	-	+	-	7.6637	3.1854	143M
Savannah	Oyo	Ilora	SW 1	-/+	-	+	+	7.7919	3.8875	293M
(DS)	Osun	Ikirun	SW 1, TC4	-/+	-	+	-	7.9434	4.6136	366M
	Ekiti	Iworoko	SW 1	-/+	-	+	-	7.7181	5.2462	412M
Lowland	Ogun	Iperu	SW 1	-/+	-	+	-	6.9802	3.5838	180M
rainforest	Oyo	Ibadan	SW 1, TC4	-/+	-	+	-	7.3025	3.3851	176M
(LR)	Osun	Osu	SW 1	-/+	-	+	-	7.5894	4.6446	341M
	Ondo	Akure	SW 1	+	+	+	-	7.3440	5.2514	359M
Fresh water	Ogun	Ado-Odo	SW 1	+	+	+	-	6.5820	2.9553	102M
swampy	Lagos	Araga-Epe	OBA S-2	+	+	+	-	6.6200	3.9631	41M
forest (FW)	Ogun	Abigi	SW 1	-/+	-	+	-	6.4868	4.3954	18M
	Ondo	Igbokoda	SW 1	-/+	-	+	-	6.3645	4.8064	17M
Mangrove	Ondo	Ugbo-nla	SW 1	-/+	-	+	-	6.1425	4.7934	4M
Forest (MF)	Lagos	Badagry	OBA S-2	+	+	+	+	6.4783	2.9179	16M
	Lagos	Aja	-	-	-	+	-	6.4717	3.5916	15M
	Lagos	Akodo	-	-	-	+	-	6.4386	3.9358	**-3M**

SW1 = Suwan – 1 - Y, TC4 = TZL Composite 4 C2, OBA S-2 = Oba Super 2, + Presence, - Absence, -/+ Irregular

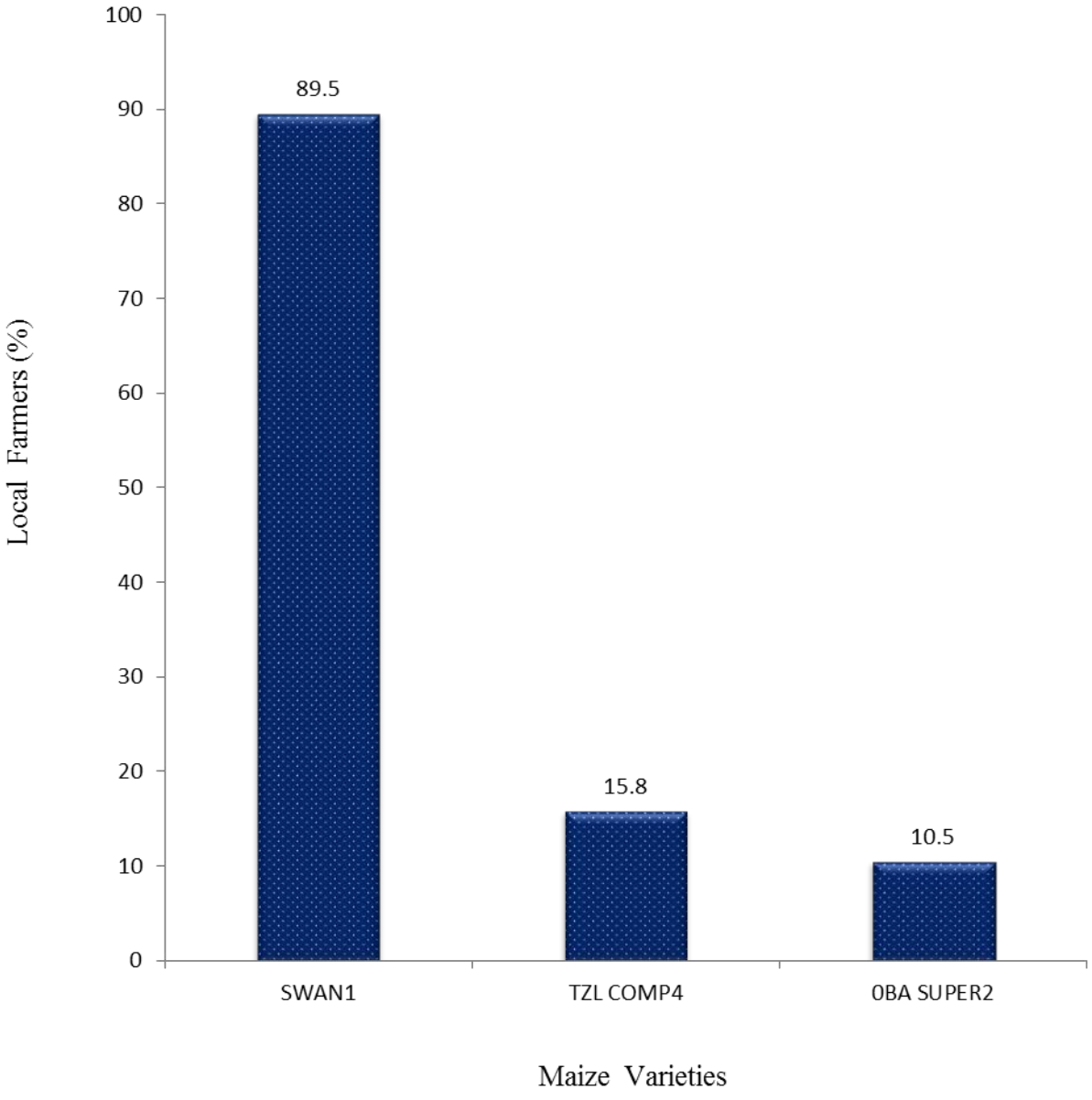


Figure 4.1: Maize varieties planted by local farmers based on study areas in Southwestern Nigeria

(6.03) and Osu (6.17) were not significantly different but were observed to be significantly ($P < 0.05$) different from soil pH of other study areas (Table 4.2). Ilora (7.00), Aja (8.00), Ibadan (7.07) and Igbokoda (7.20) were not significantly different from each other at $P < 0.05$. Table 4.2 further revealed that, the soil pH of Ado-Odo (5.07), Epe (4.73), Akodo (6.77) were significantly different ($P < 0.05$) from others compared to Akure (5.50), Abigi (5.30) and Ugbo-nla (5.37) that were as well significantly ($P < 0.05$) related. Saki (5.80) and Badagry (5.70) from two extremely different ecological zones were observed not significantly different from each other. However, the collected soil samples were predominantly slightly acidic based on the mean pH (6.18) value (Table 4.2).

The soil pH is acidic to slightly basic ranging from 4.73 to 8.00. The highest soil pH was recorded in Aja (8.00) in MF ecological zone, while the least was recorded in Epe (4.73) in FW ecological zone. Observation of soil pH in each ecological zone (Figure 4.2) showed that the soil pH of GS and MF were not significantly ($P < 0.05$) different from each other at $P < 0.05$. Therefore, observation on Saki and Badagry could as well correlate to the significant relationship of GS and MF. In addition, DS, LR and FW were also noticed to be significantly different from each other, though, GS shared significant ($P < 0.05$) relationship with LR, while MF shared significant ($P < 0.05$) relationship with DS.

Based on the moisture contents (%) of the study areas, table 4.2 revealed that the moisture contents (%) of the study areas were observed to be significantly related at $P < 0.05$. Though, the significant relationship partially varied with respect to Epe (4.35) and Saki (4.15), Badagry (1.50) and Sepeteri (1.35). The moisture contents (%) of the soil ranged from 1.35% as the lowest (Sepeteri) in GS to 4.35 as the highest (Epe) in FW. Table 4.2 also revealed sandy-clay-loam as the soil texture from OSU while Igboho, Ado-Odo, Ugbo-nla and Badagry were sand. The rest of the study areas were predominantly sandy-loam. Considering figure 4.3, there was no significant difference in the soil moisture content (%) of GS, DS and LR at $P <0.05$ compared to that of FW and MF.

Table 4.2: Physical properties of collected soil samples based on pH, moisture content (%), soil texture and particle size

Ecological zone	State	Study area	P^H	Moisture Content (%)	Texture	Particle size		
						Clay (%)	Silt (%)	Sand (%)
Guinea	Oyo	Saki	5.80 (0.00) ij	4.15 (0.1) ab	Sandy-loam	19	20	61
Savannah	Oyo	Tede	6.53 (0.05) e	2.30 (0.20) d-i	Sandy-loam	15	16	69
(GS)	Oyo	Igboho	6.03 (0.03) gh	2.00 (0.10) f-i	Sand	5	4	91
	Oyo	Sepeteri	6.37 (0.03)ef	**1.35 (0.15) i**	Sandy-loam	13	14	73
Derived	Oyo	Igangan	6.40 (0.00) e	1.80 (0.10) g-i	Sandy-loam	13	12	75
Savannah	Oyo	Ilora	7.00 (0.00) c	3.20 (0.10) a-e	Sandy-loam	11	10	79
(DS)	Osun	Ikirun	6.20 (0.00) fg	3.00 (0.20) b-f	Sandy-loam	19	14	67
	Ekiti	Iworoko	6.37 (0.03) ef	2.50 (0.40) d-i	Sandy-loam	19	6	75
Lowland	Ogun	Iperu	5.97 (0.03) hi	1.85 (0.05) f-i	Sandy-loam	17	8	75
rainforest	Oyo	Ibadan	7.07 (0.03) bc	2.65 (0.25) c-h	Sandy-loam	11	12	77
(LF)	Osun	Osu	6.17 (0.03) g	3.75 (0.15) a-c	Sandy-clay-loam	21	18	61
	Ondo	Akure	5.50 (0.00) k	2.90 (0.10) c-f	Sandy-loam	19	10	71
Fresh	Ogun	Ado-Odo	5.07 (0.03) m	2.70 (0.20) c-f	Sand	7	4	89
water	Lagos	Epe	**4.73 (0.03) n**	**4.35 (0.25) a**	Sandy-loam	19	8	73
swampy	Ogun	Abigi	5.30 (0.00) l	2.90 (0.20) c-f	Sandy-loam	15	10	75
forest(FW)	Ondo	Igbokoda	7.20 (0.06) b	2.45 (0.25) d-i	Sandy-loam	15	14	71
Mangrove	Ondo	Ugbo-nla	5.37 (0.03) kl	3.45 (0.35) a-d	Sand	5	4	91
Forest	Lagos	Badagry	5.70 (0.06) j	1.50 (0.20) hi	Sand	9	6	85
(MF)	Lagos	Aja	**8.00 (0.06) a**	1.90 (0.30) f-i	Sandy-loam	19	8	73
	Lagos	Akodo	6.77 (0.03) d	2.25 (0.15) e-i	Sandy-loam	23	7	70
Mean			6.18	2.65				
MSD ($\alpha = 0.05$)			0.19	1.19				

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences.

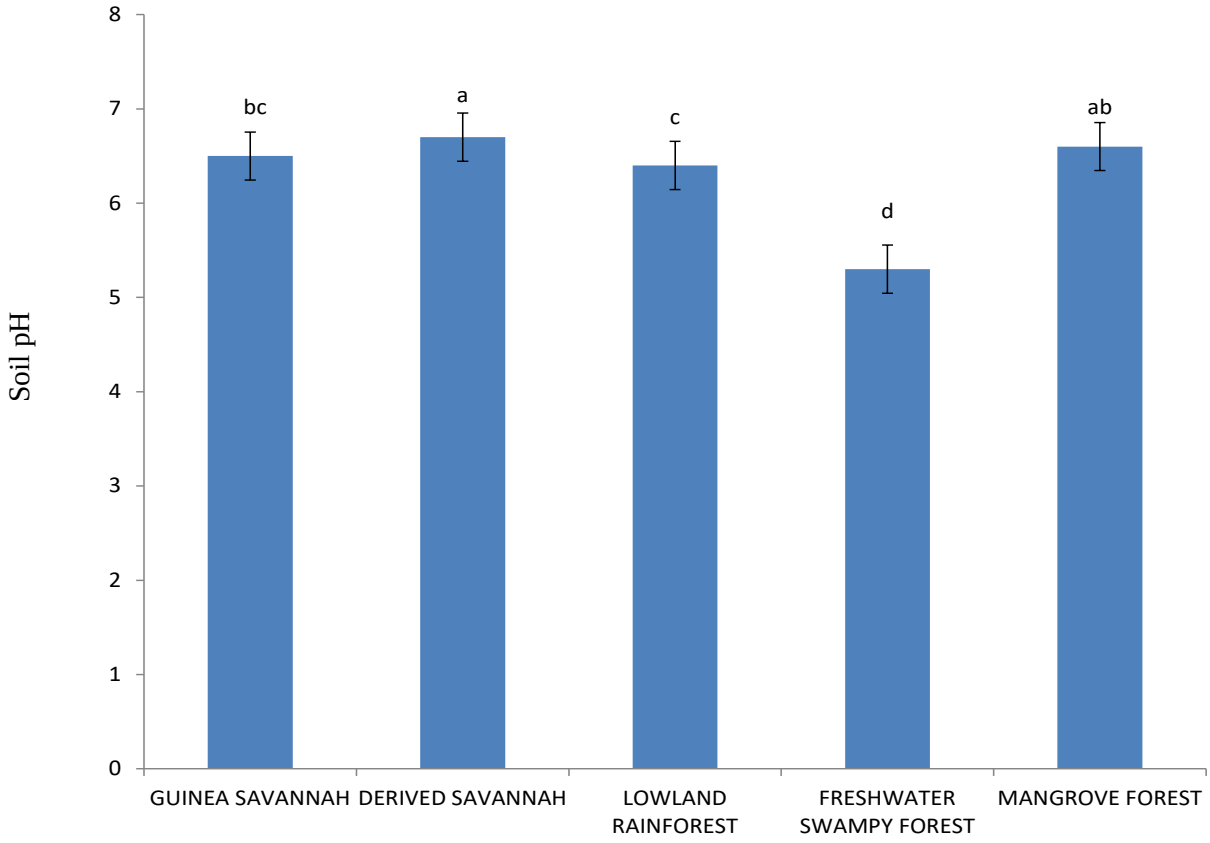


Figure 4. 2: pH of collected soil samples based on ecological zones

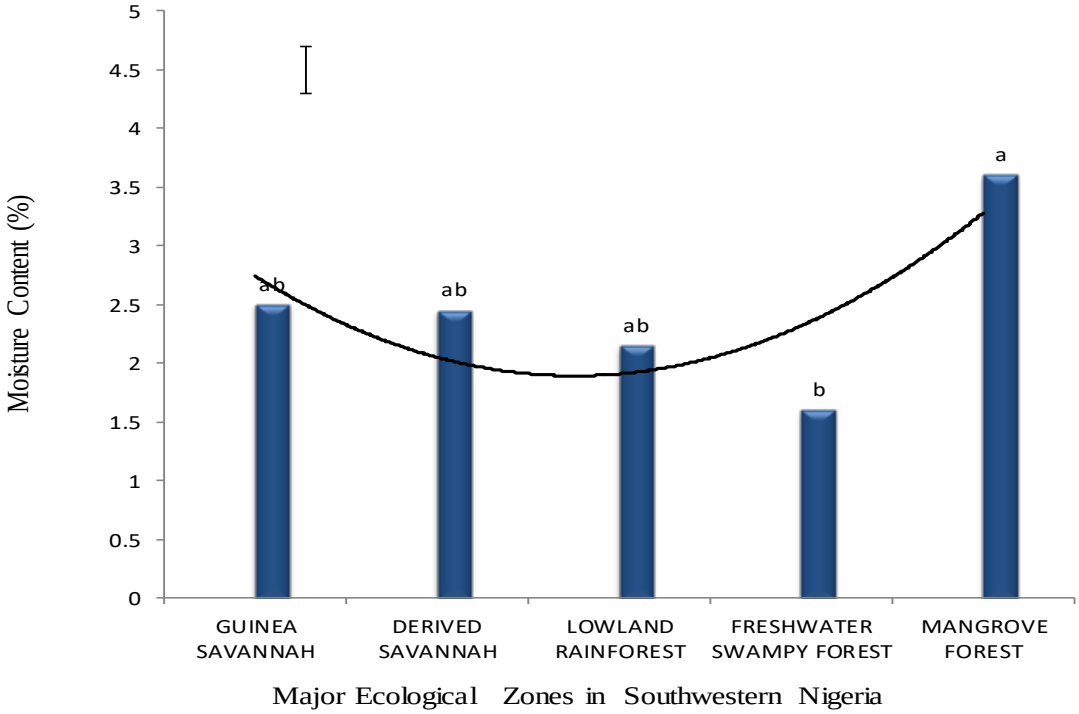


Figure 4. 3: Moisture content (%) of collected soil samples based on ecological zones

4.3 Chemical contents of collected soil samples based on the ecological zones

The chemical contents of the collected soil samples pooled together for each of the ecological zone are presented in table 4.3. Derived savannah (DS) followed by fresh water swampy forest (FW) had the highest percentage (%) of organic carbon (% OC) and Nitrogen (% N) compared to Mangrove forest (MF) that has the lowest. Lowland rainforest (LR) had a considerable % organic carbon and Nitrogen than that of Guinea Savannah (GS). Soil available phosphorus (P) (Mehlich P) in DS was observed to be extremely higher than that of other ecological zones. Available P in MF was higher than that of LR and GS, while FW had the lowest. DS had the highest exchangeable cations (Ca, Mg, K, Na, and ECEC) compared to variation observed among other ecological zones. Similarly, DS also has the highest extractable concentration of Zn and Cu while Mn and Fe were observed to be low. LR showed uniqueness with high concentration of Mn (188.17ppm), followed by GS (181.29ppm), FW (139.1ppm) and MF (46.96ppm). The concentration of Fe was generally high in all the ecological zones with the exception of DS.

4.3.1 Chemical contents of the collected soil samples based on the study areas

The organic carbon in the investigated soils was low compared to the established critical level for soil fertility in Southwestern Nigeria. The highest organic carbon was obtained from Igangan (1.90%), followed by Ibadan (1.84%), Saki, Igangan, Ilora, Ikirun, Iworoko, Ibadan, Osu and Abigi, while low organic carbon was recorded for Igboho, Sepeteri, Ugbo-nla and Aja soil compared to other study areas. The nitrogen content of the collected soil ranged from 0.04 to 0.19% (Table 4.4). The nitrogen content recorded in all the soils was relatively adequate with exception of Igboho, Sepeteri, Akure, Ugbo-nla and Aja. Available phosphorus level (Mehlich P) was relatively high in the collected soil samples, across all the study areas. Ilora had extremely high phosphorus that was 100 fold of other study areas. The potassium content of the soil ranged from 0.08 to 0.52 cmol+/kg. Iworoko had the highest potassium content (0.52 cmol+/kg), followed by Saki, Ilora and Osu, while the potassium content taken from Ado-odo, Ugbo-nla and Aja was low as shown in tabel 4.4.

Table 4.3: Chemical contents of collected soil samples based on the ecological zones

	Ecological zones				
Chemical contents	Guinea Savannah (GS)	Derived Savannah (DS)	Lowland Rainforest (LR)	Fresh Water forest (FW)	Mangrove Forest (MG)
% C	1.14	1.60	1.20	1.46	0.89
% N	0.11	0.16	0.12	0.15	0.09
Mehlich P (µg/g Soil)	17.27	177.50	27.53	14.22	37.62
Exchangeable Cations					
Ca (cmol+/ kg)	8.20	10.91	7.17	3.97	7.29
Mg (cmol+/ kg)	1.63	1.64	1.52	1.03	0.68
K (cmol+/ kg)	0.43	0.53	0.40	0.28	0.23
Na (cmol+/ kg)	0.16	0.17	0.16	0.13	0.16
ECEC (cmol+/ kg)	10.41	13.5	9.25	5.41	8.36
Extractable micronutrients(ppm)					
Zn (ppm)	33.30	85.87	44.45	21.72	37.37
Cu (ppm)	1.27	44.45	1.60	0.62	0.60
Mn (ppm)	181.29	21.72	188.17	139.1	46.96
Fe (ppm)	144.31	37.37	163.14	147.92	182.70

Guinea Savannah (GS), Derived Savannah (DS), Lowland Rainforest (LR), Fresh water forest (FW), Mangrove Forest (MF). % C = Percentage of carbon, % N = percentage of nitrogen, P = Phosphorus, Ca = Calcium, Mg = Magnesium, K = Potassium, Na = Sodium, Zn = Zinc, Cu = Copper, Mn = Manganese, Fe = Iron.

Observation in table 4.4 on exchangeable cations (cmol+/kg) showed that, calcium content of the investigated soil samples ranged from 2.35 to 10.92cmol+/kg. Tede had the highest (10.92cmol+/kg) calcium content. Although, the calcium content observed in the collected soil samples across the study areas were in line to the expected standard (>1.6) but Igboho, Epe and Ondo had low calcium content compared to other study areas. Further observation showed that, the magnesium content ranged from 0.24 to 2.21 cmol+/kg. The magnesium in the soil samples was comparatively adequate with the exception of Igboho, Akure, Epe and study areas in mangrove forest that were relatively not encouraging.

The highest sodium content was recorded for Tede (0.17 cmol+/kg) and Ilora (0.17 cmol+/kg), while the least was obtained from soil samples collected from Epe. Similarly, Exchangeable cations exchange capacity (ECEC) was highest in sample taken from Ilora (16.26 cmol+/kg), followed by Tede (13.48 cmol+/kg), while the least ECEC value was recorded for Ugbo-nla (3.38cmol+/kg) soil. Akodo (105.67 ppm) had the highest zinc (Zn) content, followed by Ilora (104.71 ppm), while the lowest Zn content was obtained from Saki (9.00 ppm) soil. Copper (Cu) content in the investigated soil was adequate. Although, the copper content obtained in mangrove forest ecological zone of the study areas were generally low compared to other ecological zones. Iperu showed uniqueness with highest (1.95 ppm) copper content. The values of iron (Fe) and manganese (Mn) obtained from the collected soil samples across the study areas including Igboho were observed to be extremely high (Table 4.4).

4.4. Bacteria load of collected soil samples based on the source (soil / rhizosphere) of collection

Interestingly, both the highest (65.50 x 10^{-6} CFU / g) and the lowest bacteria load (4.00 x 10^{-6} CFU / g) were from Saki and Tede respectively, justifying the implication of the same ecological zone (Derived savannah). The same significant ($P < 0.05$) similarity in the study areas for the maize rhizosphere was also observed for the collected soil samples from the study areas. Although, the bacteria load from Tede (190.00 x 10^{-6} CFU / g) study area statistically proofed to be significantly ($P < 0.05$) different from other study areas. Two extreme ecological zones were observed to harbor both the highest (190 x 10^{-6} CFU / g) and the lowest (2.50 x 10^{-0} CFU / g) from Tede (guinea savannah) and Badagry (mangrove forest) respectively (Table 4.5).

Table 4.4: Chemical contents of collected soil samples based on study areas in each ecological zone

Eco zones	State	Study area	% OC	% N	Mehlich P (µg/gsoil)	Exchangeable Cations (cmol+/kg)					Extractable micronutrients (ppm)			
						Ca	Mg	K	Na	ECEC	Zn	Cu	Mn	Fe
GS	Oyo	Saki	1.39	0.14	3.85	6.23	1.23	0.45	0.15	8.06	9.00	0.41	181.71	116.41
	Oyo	Tede	1.46	0.13	55.06	10.92	2.07	0.32	0.17	13.48	81.05	0.63	143.45	138.84
	Oyo	Igboho	0.75	0.04	15.70	2.95	0.24	0.08	0.13	3.40	37.30	0.25	9.86	95.04
	Oyo	Sepeteri	0.98	0.06	4.87	5.29	1.09	0.28	0.15	6.81	16.24	1.12	170.79	123.51
DS	Oyo	Igangan	1.90	0.19	6.57	8.73	1.53	0.4	0.16	10.83	18.60	0.93	182.13	150.23
	Oyo	Ilora	1.54	0.15	398.83	14.01	1.62	0.45	0.17	16.26	104.71	1.47	182.13	175.60
	Osun	Ikirun	1.44	0.14	19.07	8.16	1.85	0.44	0.16	10.60	38.82	1.60	195.41	151.47
	Ekiti	Iworoko	1.36	0.14	16.50	5.32	1.07	0.52	0.14	7.05	29.57	0.69	171.93	142.56
LR	Ogun	Iperu	1.26	0.11	10.74	6.33	1.67	0.21	0.15	8.36	46.87	1.95	187.79	125.76
	Oyo	Ibadan	1.84	0.18	71.23	8.12	1.44	0.34	0.16	10.06	67.25	1.19	139.28	183.38
	Osun	Osu	1.55	0.15	16.20	8.95	2.21	0.48	0.16	11.80	41.56	1.76	184.73	137.38
	Ondo	Akure	1.03	0.09	12.03	4.75	0.74	0.26	0.14	5.89	30.77	0.93	195.10	162.80
FW	Ogun	Ado-Odo	1.09	0.1	4.66	3.30	1.23	0.19	0.14	4.86	14.50	1.09	165.74	122.83
	Lagos	Epe	1.30	0.13	14.83	2.89	0.88	0.31	0.12	4.28	15.54	0.71	157.70	165.34
	Ogun	Abigi	1.56	0.14	19.77	4.96	1.38	0.30	0.14	6.79	35.27	0.60	84.17	157.95
	Ondo	Igbokoda	1.22	0.10	10.40	6.76	1.34	0.40	0.15	8.66	26.06	1.24	184.73	128.36
MF	Ondo	Ugbo-nla	0.56	0.06	5.05	2.35	0.74	0.16	0.13	3.38	15.25	0.26	60.98	194.54
	Lagos	Badagry	1.32	0.13	58.32	4.48	0.91	0.36	0.14	5.88	16.35	0.25	71.39	145.66
	Lagos	Aja	0.74	0.04	8.70	9.09	0.54	0.19	0.16	9.97	22.06	0.54	14.00	167.71
	Lagos	Akodo	1.06	0.11	86.32	8.15	0.52	0.23	0.16	9.06	105.67	0.16	30.85	293.99

Acronyms for ecological zones and chemical contents are defined in Table 2.

Table 4.5: Bacteria load based on source of collection (Rhizosphere / Soil) at 10^{-6} CFU/g soil

		10^{-6} CFU/g soil	
Ecological zone	Study area	Rhizosphere	Soil
GS	Saki	**65.50 (5.5) a**	19.00 (6.00)e-g
	Tede	**0.00 (0.00) h**	**190.00 (9.00) a**
	Igboho	40.50 (8.00) c-h	13.50 (1.50) gf
	Sepeteri	11.50 (2.5) gh	30.00 (2.00) d-g
DS	Igangan	80.00 (12.00) a-c	19.00 (3.00) e-g
	Ilora	24.50 (3.50) e-h	58.50 (9.50) c-e
	Ikirun	13.00 (4.00) gh	31.00 (4.00) d-g
	Iworoko	98.00 (4.50) a	131.00 (2.00) b
LR	Iperu	71.00 (7.00) a-d	47.03 (6.00) d-f
	Ibadan	40.50 (8.50) c-h	25.00 (6.00) e-g
	Osu	29.50 (3.50) e-h	25.00 (2.00) e-g
	Akure	19.00 (2.00) f-h	72.50 (11.50) cd
FW	Ado-Odo	29.00 (12.00) e-h	91.50 (9.50) bc
	Araga-Epe	25.00 (6.00) e-h	36.00 (23.00) d-g
	Abigi	54.50 (13.50) b-f	50.00 (7.00) c-f
	Igbokoda	45.50 (7.50) c-g	15.00 (2.00) e-g
MF	Ugbo-nla	43.00 (6.00) c-g	29.00 (4.000 d-g
	Badagry	30.50 (10.50) d-h	**2.50 (1.50) g**
	Aja	92.00 (3.00) ab	72.00 (3.00) cd
	Akodo	16.00 (5.00) f-h	16.00 (5.00) e-g
	Mean	41.35	48.68
	MSD($\alpha = 0.05$)	41.15	44.28

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. CFU = Colony Forming Unit. Acronyms for ecological zones are defined in Table 2.

Table 4.6: Primary and Post – Primary antagonistic screening of isolates

Sample Code	Number of Isolates	(Primary screening) Number of antagonistic isolates	(Post – Primary screening) Number of antagonistic isolates
SKR	10	6	3
SKS	8	1	1
TDR	9	6	1
TDS	11	8	1
IGR	15	3	1
IGS	21	11	0
SPR	13	5	0
SPS	11	6	1
IGGR	21	12	3
IGGS	13	6	0
ILR	10	5	2
ILS	15	13	2
IKR	11	3	2
IKS	18	2	1
IWR	11	10	1
IWS	`0	2	1
IBR	8	7	1
IBS	12	8	1
IPR	10	7	3
IPS	9	4	0
OSR	17	10	2
OSS	9	3	0
AKUR	13	4	0
AKUS	8	2	0
ADR	12	3	0
ADS	18	9	1
EPR	13	5	5
EPS	12	5	1
ABR	10	4	0
ABS	13	7	2
IGBR	14	9	1
IGBS	12	3	0
UNR	11	5	1
UNS	12	3	2
EBR	15	9	2
EBS	9	5	1
AJR	13	3	0
AJS	15	5	1
AKR	11	8	3
AKS	10	10	1
Total	**445(100%)**	**237 (48.67%)**	**48 (20.25%)**

Table 4.7: Distribution of antagonistic soil bacteria isolate (s) based on source of collection and study area per ecological zone.

Study area	**Number of antagonistic 1solates**		**Isolate code**
	Rhizosphere	Soil	
SAKI	3	1	SKR2, SKR5, AT-SKR, SKS3
TEDE	1	1	TDR6, TDS9
IGBOHO	1	0	IGR1
SEPETERI	0	1	SPS1
IGANGAN	3	0	IGGR5, IGGR8, IGGR11
ILORA	2	2	ILR6, AT-ILR, ILS13, ILS14
IKIRUN	2	1	IKR1, IKR11, AT-IKS
IWOROKO	1	1	IWR2, IWS1
IBADAN	1	1	IBR6, IBS8
IPERU	3	0	IPR1, IPR2, IPR5
OSU	2	0	OSR7, OSR10
AKURE	0	0	
ADO-ODO	0	1	ADS 14
EPE	5	1	EPR 1-4, EPRR 7, AT-EPS
ABIGI	0	2	ABS 6, ABS 8
IGBOKODA	1	0	IGBR 11
UGBO-NLA	1	2	UNR 3, UNS 8, UNS 9
BADAGRY	2	1	EBR 1, EBR 4, EBS 8
AJA	0	1	AJS 2
AKODO	3	1	AKR 2, AKR 5, AKR 8, AKS 2
TOTAL	31 (64.58%)	17 (35.42%)	

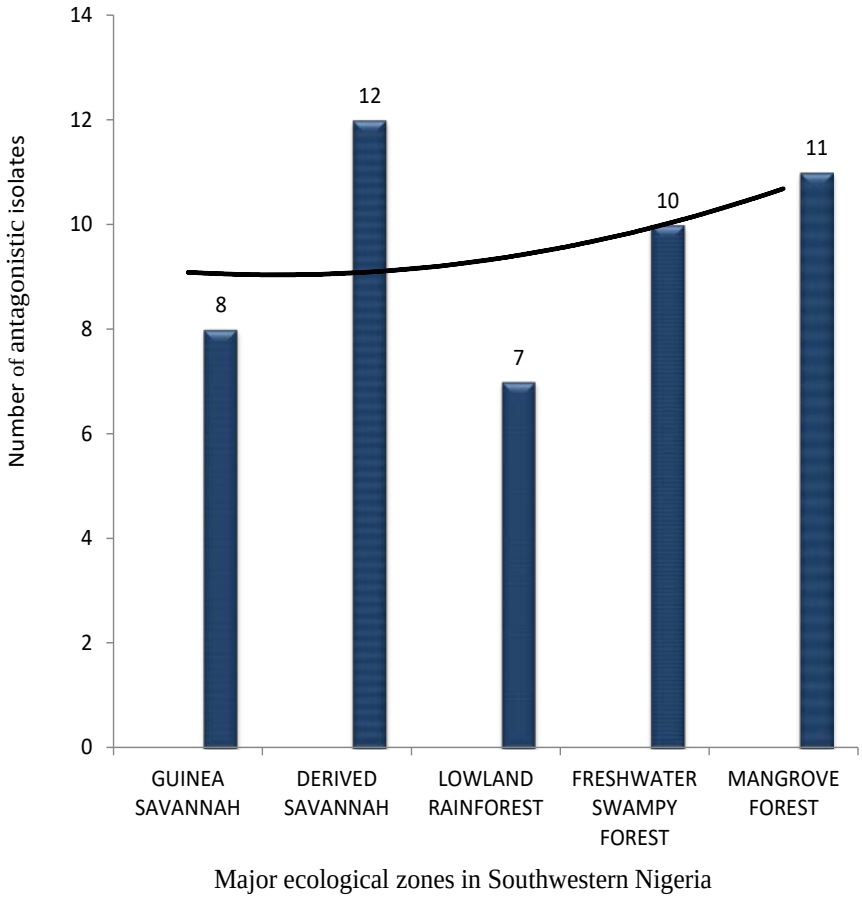


Figure 4.4: Total number of antagonistic bacteria isolates based on each ecological zone

Plate 4.1: Antagonist – pathogen interaction at day 15 (In – vitro)

4.5 Antagonistic potentials of bacteria isolates

Out of four hundred and fourtyfive (445) isolates, two hundred and thirtyseven (237) were antagonistic (in-vitro) to pathogenic *Fusarium verticillioides* (Table 4.6). Re-confirmation (Plate 4.1) of isolates antagonistic potentials justified that only fourty eight (48) were actually antagonistic. Table 4.7 revealed that isolates from Saki (SKR2, SKR5 and AT-SKR), Igangan (IGGR5, IGGR8 and IGGR11), and Akodo (AKR2, AKR5 and AKR8) study areas were observed to have the same number of antagonistic isolates with respect to rhizosphere as the source of collection. Epe (EPR1, EPR2, EPR3, EPR4 and EPR7) study areas had the highest antagonistic bacteria isolates from rhizosphere of maize, while none was recorded for Sepeteri, Akure, Ado-Odo, Abigi and Aja (Table 4.7). However, antagonistic bacteria isolates were less predominates in collected soil samples from the study areas compared to that of maize rhizosphere (Table 4.7). Based on the ecological zones, DS had the highest antagonistic bacteria followed by MF, FW and GS while the least was observed in LR (Figure 4.4).

4.6 Plant growth promoting characteristics of bacteria isolates

Exactly 61.20% of the treatments significantly solubilize phosphate. Interestingly, AKR5 treatment had the highest phosphate solubilization efficiency (68.34%), though, not significantly ($P < 0.05$) different from AT-ILR, IGGR11, IPR2, AKS2, EPR3, and AKR8 but significantly ($P < 0.05$) different from other treatments. Also, ILS13, ILR6, EPR1, IPR1, IGR1, AJS2, AKR2, IWS1, EBR1 and SKS3 were observed with related solubilization phosphate efficiency. Other treatments were not significantly different from the control (Table 4.8). Exactly 63.3% of the bacteria isolates significantly showed halozone in the replicated inoculated plates, signifying chitinase production. Highest chitinase activity was from IGR1 and was observed not significantly different ($P < 0.05$) from EPR2 and IBS8. Other bacteria isolates were also not really significantly ($P < 0.05$) different from each other in their chitinase activity (Table 4.8).

The spectrophotometer readings of existing indole rings for each isolate was justified in table 4.12. Production of Indole – 3- Acetic Acid (IAA) significantly ($P < 0.05$) varied across the treatments. Although, the quantity produced by 20% of the treatments were relatively low but would be advantageous to enhance maize growth. When L-tryptophan was added to the medium, the amount of IAA measured, produced by the treatment (bacteria) was tolerated between 1.00 and 28.24mg/l. Treatment EBR1 exuded the highest (28.24mg/l) of IAA, followed by treatment

ILS13 (26.79mg/l). Similarly, the quantity of IAA produced by AT-SKR, EPS, AT-IKS, ILR6, AJS2, AKR2, SKS3, IBR6, IKR1, ILS14, IPR5 and UNR3 were relatively high, thus, would enhance maize growth (Table 4.9).

When D-L tryptophan was added to the medium, significant ($P < 0.05$) differences were observed in IAA produced by the treatments. The IAA produced by the treatments ranged from 2.28 to 33.20mg/l. AT-SKR produced the highest (33.20mg/l) IAA, followed by AJS2 (32.50mg/l). Moreso, ANOVA showed that the IAA produced by the treatment AT-EPS, SKS3 and ADS14 were significantly ($P < 0.05$) similar but were observed to be significantly($P < 0.05$) different from IAA produced by treatment AKR8, EPR2, EPR7, UNS8, AT-IKS, ILS13, EPR1, IWS1, EBR1, IBR6, IGGR11, IGGR5, OSR10, SKR2, IGGR8, IPR5, TDR6, OSR7 and other treatments. Generally, observation revealed that metabolism of D-L tryptophan significantly encouraged production of IAA more than the L- tryptophan (Table 4.9).

All the treatments enhanced maize seed germination in the laboratory but were observed to be significantly ($P < 0.05$) similar in their percentage seed germination (Table 4.10). Not less than 60.42% of the treatments significantly ($P < 0.05$) increased radicle length, while 47.92% of the treatments caused plumule length to increase (Table 4.11). AT-IKS gave the highest (8.09) radicle length, followed by EBS8 (8.02) and EPR3 gave the lowest (3.24) radicle length. The highest plumule length was recorded for ADS14, followed by EBR4 (5.95), while the lowest (0.37) plumule length was obtained from UNS9 treatment (Table 4.11).

4.7 Antagonist-pathogen interaction effect on maize seed germination

Effect of antagonist-pathogen interaction on maize seed germination is presented in table 4.12. In the positive control experiment (MZ + Pt), the pathogen significantly ($P < 0.05$) inhibited the germination of maize to seedling level with diverse symptoms of diseases such as leaf curl, leaf blight and stem rot. Over 70% of the treatments significantly ($P< 0.05$) control the pathogen. Interestingly, AKS2, AKR8, EPR7, AKR2, EBR1, SKS3, ABS8 and TDR6 significantly ($P < 0.05$) had 100% maize seed germination compared to other treatments. AKR5, EBR4, EPR1, IPR1, IGR1, ILS14, IGGR5, SKR2, IGGR8 and OSR7 were significantly similar to the negative control (MZ alone) in their performance to enhance maize seed germination during antagonist-pathogen interaction.

Table 4.8: Potentials of soil bacteria isolates to solubilize phosphate and produce chitinase (In-vitro)

S/N	Treatment	Phosphate (%)	Chitinase (%)	S/N	Treatment	Phosphate	Chitinase
0	CONTROL	0.00 (0.00) i	0.00 (0.00) i	25	AKR2	10.80 (1.71) g-i	1.50 (0.20) i
1	AKR5	**68.34 (1.67) a**	1.40 (0.00) i	26	IWS1	10.03 (0.51) g-i	0.00 (0.00) i
2	AT-ILR	52.41 (11.23) ba	2.10 (0.00) i	27	EBR1	9.35 (0.66) g-i	0.00 (0.00) i
3	IGBR11	51.91 (1.93) a-c	1.40 (0.20) i	28	SKS3	8.23 (1.78) g-i	59.28 (5.43) bc
4	IPR2	50.59 (9.41) a-d	0.00 (0.00) i	29	ADS14	6.27 (0.40) hi	41.67 (8.34) c-e
5	AKS2	48.34 (1.67) a-d	1.60 (0.10) i	30	IBR6	4.12 (0.42) hi	11.07 (2.00) hi
6	EPR3	45.30 (0.86) a-e	13.89 (2.78) g-i	31	ABS6	0.00 (0.00) i	0.00 (0.00) i
7	AKR8	44.95 (0.51) a-f	0.00 (0.00) i	32	IGGR11	0.00 (0.00) i	0.00 (0.00) i
8	EPR4	42.50 (7.50) b-f	25.11 (2.89) e-h	33	IKR1	0.00 (0.00) i	0.00 (0.00) i
9	AT-EPS	41.67 (8.34) b-f	2.70 (0.40) i	34	ILS14	0.00 (0.00) i	34.31 (0.98) e-f
10	EBR4	41.27 (3.18) b-f	1.25 (0.05) i	35	IGGR5	0.00 (0.00) i	0.00 (0.00) i
11	EPR2	41.26 (4.90) b-f	67.92 (5.42) ab	36	IWR2	0.00 (0.00) i	0.00 (0.00) i
12	EBS8	33.64 (10.12) b-g	1.50 (0.30) i	37	ABS8	0.00 (0.00) i	0.00 (0.00) i
13	SPS1	20.09 (10.91) b-h	0.00 (0.00) i	38	OSR10	0.00 (0.00) i	0.00 (0.00) i
14	EPR7	26.18 (10.18) c-h	11.80 (3.20) hi	39	IKR11	0.00 (0.00) i	22.47 (0.25) f-h
15	UNS8	25.76 (7.58) d-i	2.00 (0.20) i	40	SKR2	0.00 (0.00) i	63.06 (1.95) ab
16	AT-SKR	22.22 (7.41) e-i	1.35 (0.05) i	41	SKR5	0.00 (0.00) i	27.19 (6.14) e-h
17	IBS8	19.66 (3.87)e-i	51.67 (1.67) b-d	42	IGGR8	0.00 (0.00) i	30.07 (6.99) e-g
18	AT-IKS	19.32 (5.69) f-i	1.70 (0.10) i	43	IPR5	0.00 (0.00) i	59.06 (5.20) bc
19	ILS13	13.04 (2.34) g-i	0.00 (0.00) i	44	TDR6	0.00 (0.00) i	12.88 (3.79) g-i
20	ILR6	12.25 (0.25) g-i	0.00 (0.00) i	45	TDS9	0.00 (0.00) i	0.00 (0.00) i
21	EPR1	12.14 (2.14) g-i	25.87 (4.13) e-h	46	UNR3	0.00 (0.00) i	0.00 (0.00) i
22	IPR1	11.91 (2.39) g-i	10.55 (1.95) hi	47	OSR7	0.00 (0.00) i	0.00 (0.00) i
23	IGR1	11.86 (3.29) g-i	**80.84 (10.84) a**	48	UNS9	0.00 (0.00) i	1.20 (0.10) i
24	AJS2	11.56 (0.45) g-i	1.65 (0.25) i		LSD ($\alpha = 0.05$)	25.81	17.86

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences.

Table 4.9: In-vitro production of Indole – 3- acetic acid (IAA)

S/N	Treatments	L-Trytophan	D-L Tryptophan	S/N	Treatments	L-Trytophan	D-L Tryptophan
0	CONTROL	0.00 (0.00) a	0.00 (0.00) k	25	AKR2	23.82 (1.18) bc	7.75 (0.05) gh
1	AKR5	7.43 (0.12) p-s	5.54 (0.03) g-j	26	IWS1	14.40 (0.40) i-k	15.75 (0.15) de
2	AT-ILR	4.87 (0.01) t-w	8.55 (0.35)g	27	EBR1	**28.24 (0.33) a**	16.22 (0.22) de
3	IGBR11	7.34 (0.22) p-s	3.77 (0.33) h-j	28	SKS3	18.20 (0.60) fg	25.60 (0.10) b
4	IPR2	3.60 (0.60) u-y	4.60 (0.30) g-j	29	ADS14	12.70 (0.20) kl	24.60 (0.10) b
5	AKS2	1.00 (0.10) a-z	2.30 (0.20) jk	30	IBR6	22.05 (1.05) d	14.50 (0.50) ef
6	EPR3	10.35 (0.35) m-o	2.83 (0.08) jk	31	ABS6	2.05 (0.06) yz	11.40 (0.40) f
7	AKR8	9.31 (0.19) n-p	14.45 (0.45) ef	32	IGGR11	10.50 (0.40) mn	13.76 (0.23) ef
8	EPR4	6.20 (0.10) q-t	5.20 (0.20) g-j	33	IKR1	19.59 (0.29) ef	7.59 (0.29) gh
9	AT-EPS	16.58 (0.20) gh	26.18 (2.73) b	34	ILS14	19.25 (0.25) ef	11.70 (0.20) f
10	EBR4	3.51 (0.41) u-y	4.74 (0.24) g-j	35	IGGR5	2.76 (0.15) w-z	16.60 (0.30) de
11	EPR2	6.25 (0.25) q-t	15.70 (0.20) de	36	IWR2	4.55 (0.35) t-x	2.42 (0.08) jk
12	EBS8	5.27 (0.06) s-v	4.77 (0.12) g-j	37	ABS8	8.31 (0.20) o-q	7.65 (4.50) gh
13	SPS1	7.81 (0.11) p-r	7.50 (0.40) gh	38	OSR10	3.67 (0.30) u-y	18.53 (0.23) d
14	EPR7	15.70 (0.20) hi	14.65 (0.25) d-f	39	IKR11	3.60 (0.30) u-y	3.58 (0.13) ij
15	UNS8	8.50 (0.41) n-q	15.65 (0.25) de	40	SKR2	7.30 (0.20) p-s	21.80 (0.30) c
16	AT-SKR	11.40 (0.40) lm	**33.20 (0.90) a**	41	SKR5	8.15 (0.15) pg	4.71 (0.23) g-j
17	IBS8	4.65 (0.15) t-w	2.75 (0.15) jk	42	IGGR8	14.65 (0.35) h-j	18.55 (0.05) d
18	AT-IKS	17.70 (0.20) fg	18.47 (0.48) d	43	IPR5	22.65 (0.25) cd	14.84 (0.06) d-f
19	ILS13	26.79 (0.79) a	12.79 (0.10) ef	44	TDR6	9.40 (0.18) n-p	15.64 (0.34) de
20	ILR6	20.90 (1.10) de	2.28 (0.28) jk	45	TDS9	5.75 (0.15) r-u	7.62 (0.28) gh
21	EPR1	13.67 (0.10) jk	18.54 (0.44) d	46	UNR3	25.15 (2.25) b	4.59 (0.19) g-j
22	IPR1	3.57 (0.42) u-y	6.70 (0.20) g-i	47	OSR7	2.40 (0.40) x-z	16.50 (0.40) de
23	IGR1	7.40 (0.42) p-s	7.65 (0.15) gh	48	UNS9	3.26 (0.16) v-y	7.17 (0.17) g-i
24	AJS2	16.49 (0.29) gh	32.50 (0.40) a		LSD ($\alpha = 0.05$)	1.46	2.27

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences.

Table 4.10: Effect of bacteria isolates on maize seed germination in the laboratory

S/N	Treatments	Maize seed germination (%)	S/N	Treatments	Maize seed germination (%)
0	CONTROL	100.00 (5.00) a	25	AKR2	95.00 (5.00) a
1	AKR5	95.00 (0.00) a	26	IWS1	100.00 (0.00) a
2	AT-ILR	85.00 (10.00) a	27	EBR1	95.00 (5.00) a
3	IGBR11	95.00 (5.00) a	28	SKS3	85.00 (5.00) a
4	IPR2	95.00 (0.00) a	29	ADS14	90.00 (0.00) a
5	AKS2	95.00 (0.00) a	30	IBR6	95.00 (0.00) a
6	EPR3	85.00 (10.00) a	31	ABS6	100.00 (0.00) a
7	AKR8	100.00 (0.00) a	32	IGGR11	100.00 (0.00) a
8	EPR4	100.00 (10.00) a	33	IKR1	95.00 (0.00) a
9	AT-EPS	95.00 (0.00) a	34	ILS14	95.00 (0.00) a
10	EBR4	100.00 (0.00) a	35	IGGR5	95.00 (10.00) a
11	EPR2	95.00 (0.00) a	36	IWR2	85.00 (5.00) a
12	EBS8	95.00 (0.00) a	37	ABS8	100.00 (0.00) a
13	SPS1	95.00 (5.00) a	38	OSR10	100.00 (0.00) a
14	EPR7	95.00 (5.00) a	39	IKR11	95.00 (5.00) a
15	UNS8	100.00 (0.00) a	40	SKR2	95.00 (10.00) a
16	AT-SKR	85.00 (15.00) a	41	SKR5	95.00 (5.00) a
17	IBS8	95.00 (5.00) a	42	IGGR8	95.00 (10.00) a
18	AT-IKS	100.00 (0.00) a	43	IPR5	95.00 (5.00) a
19	ILS13	100.00 (5.00) a	44	TDR6	95.00 (5.00) a
20	ILR6	95.00 (0.00) a	45	TDS9	95.00 (0.00) a
21	EPR1	100.00 (0.00) a	46	UNR3	95.00 (5.00) a
22	IPR1	100.00 (0.00) a	47	OSR7	100.00 (0.00) a
23	IGR1	100.00 (5.00) a	48	UNS9	75.00 (5.00) a
24	AJS2	95.00 (5.00) a		LSD ($\alpha = 0.05$)	33.26

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences.

Maize diseases such as leaf curl, leaf blight and stem rot were physically expressed on maize plants treated with fungal pathogen (MZ + Pt) at day 14. However, maize diseases were not significantly ($P < 0.05$) expressed in maize plants co-treated with bacteria and fungal pathogen at day 14. Table 4.13 showed that AT-ILR, EPR3, EBS8, ADS14, IGGR11, IKR1, IWR2, SKR5, IGGR8 and OSR7 were significantly ($P < 0.05$) similar to each other with percentage disease expression of 11%. EPR2 and SKR2 showed 26.47% and 25.00% disease expression respectively. Maize plant treated with the fungal pathogen and treatment UNS8 and IPR5 manifested 30.53% and 30.00% disease expression respectively. Further observation in table 4.13 showed that maize plant treated with UNR3 in the presence of the fungal pathogen revealed 42.90% disease expression on the maize plant. At day 21, 43.75% of the treatments showed symptoms significantly ($P < 0.05$) similar to the positive control (MZ + Pt). Disease expression on mazie plants across the treatments were significantly ($P < 0.05$) similar, though with sligth significant ($P < 0.05$) variation. Interestingly, disease expressions were delayed between day 14 and day 21. That is, the percentage of disease expression was decreased from 87.76% at day 14 to 56.25% at day 21 which showed strong indication that 31.50% of the treatments were able to antagonize the pathogenic fungi within the period of 7days, therefore, increased the chances of some of the treatments as biocontrol agents. Treatment IPR5 was significantly ($P < 0.05$) not effective because the disease expression on maize plants were physically obvious and similar to that of positive control (MZ + Pt). AKS2, AKR8, EPR7, AKR2, EBR1, SKS3, ABS8, IKR11, TDR6 and TDS9 were observed to have acted as biocontrol agents as no traces of diseases were physically observed on all the replicated maize plants. Generally, maize disease expression at day 21 significantly varied. There was no significant disease expression on the negative control (MZ alone).

4.7.1 Antagonist-pathogen interaction effects on maize plant height and stem girth.

The positive control (MZ + Pt) significantly ($P < 0.05$) inhibited maize plant height compared to the negative control (MZ alone) that was significantly ($P < 0.05$) shorter. All the treatments significantly ($P < 0.05$) produced maize plants with very good height. Observation revealed in table 4.14 that the plant height for the treatments were not significantly ($P < 0.05$) different from each other but significantly ($P < 0.05$) different from the positive control (MZ + Pt). The maize

plant height ranged from 22.20 cm as the highest for TDR6 treatment to the 16.83 cm as the lowest for SPS1 treatment. Observation in table 4.18 revealed the effect of treatments on stem girth. The effect of treatments on stem girth was significantly ($P < 0.05$) discouraged. Significant similarities were observed on stem girth of all the treatments including controls.

4.7.2Antagonist-pathogen interaction effects on maize plant number of leaves and leaf areas.

Leaf number of maize plant involving positive control (MZ + Pt) and negative control (MZ alone) were significantly ($P < 0.05$) different when compared (Table 4.19). Surprisingly, there were no significant ($P < 0.05$) differences in leaf number across the treatments. The leaf areas of the treated maize plants were significantly ($P < 0.05$) similar, only with the exception of positive control (MZ + Pt). The highest (122.75 cm^2) leaf area was recorded for OSR7 treatment while the lowest (60.93 cm^2) was recorded for AKR8 treated maize plant (Table 4.15 and Table 4.16).

4.7.3 Antagonist-pathogen interaction effects on maize plant rhizosphere bacterial load.

The bacteria load significantly ($P < 0.05$) varied across the treatments. The bacteria load was higher in all the treatments with the exception of negative control (MZ alone), positive control (MZ + Pt) that was extremely low, AKR5, AKR8, EBS8, IGGR11, SKR2 and OSR7 (Table 4.17). The bacteria load of treatment AT-ILR, IPR2, AKS2, EPR3, EPR4, AT-EPS, EBR4, IBS8, AT-IKS, IPR1, ADS14, ILS14, IKR11, IPR5 and TDS9 were observed to be higher compared to other treatments. Generally, the bacteria load of the treatments significantly ($P < 0.05$) increased in maize rhizosphere compared to the controls.

Data on plant growth promoting characteristics of isolates, disease expression percentage, and biocontrol activities of isolates as well as the maize plant morphological parameters were co-

Table 4.11: Effect of bacteria isolates on radicle and plumule length during germination

S/N	Treatments	Radicle length (cm)	Plumule length (cm)	S/N	Treatments	Radicle Length (cm)	Plumule length (cm)
0	CONTROL	5.77 (0.54) a-g	2.07 (0.10) a-j	25	AKR2	5.18 (0.75) a-g	1.10 (0.77) d-j
1	AKR5	3.71 (0.50) e-g	0.75 (0.22) h-j	26	IWS1	5.97 (0.77) a-g	2.32 (0.29) a-i
2	AT-ILR	7.09 (1.19) a-e	2.13 (0.10) a-j	27	EBR1	6.08 (0.45) a-g	2.88 (0.45) a-e
3	IGBR11	5.55 (0.48) a-g	1.22 (0.15) b-j	28	SKS3	3.38 (0.15) fg	0.84 (0.37) g-j
4	IPR2	4.43 (0.70) b-g	1.09 (0.29) d-j	29	ADS14	7.74 (0.64) a-c	3.27 (0.17) a
5	AKS2	4.59 (0.02) a-g	0.81 (0.04) h-j	30	IBR6	7.04 (0.57) a-e	2.30 (0.47) a-i
6	EPR3	3.24 (0.54) g	0.59 (0.29) ij	31	ABS6	7.42 (0.49) a-d	2.30 (0.27) a-i
7	AKR8	5.05 (0.58) a-g	2.35 (0.35) a-i	32	IGGR11	7.49 (0.09) a-d	2.70 (0.53) a-f
8	EPR4	4.50 (0.54) a-g	0.96 (0.69) f-j	33	IKR1	6.58 (0.05) a-g	2.50 (0.00) a-h
9	AT-EPS	6.58 (0.05) a-g	2.50 (0.00) a-h	34	ILS14	5.52 (0.09) a-g	2.39 (0.32) a-i
10	EBR4	5.95 (0.42) a-g	3.05 (0.05) ab	35	IGGR5	7.32 (0.09) a-d	2.39 (0.32) a-i
11	EPR2	3.90 (0.20) d-g	1.35 (0.05) b-j	36	IWR2	6.57 (0.17) a-g	2.13 (0.44) a-j
12	EBS8	8.02 (0.75) ab	2.92 (0.19) a-d	37	ABS8	4.52 (0.45) a-g	1.62 (0.39) a-j
13	SPS1	5.40 (0.07) a-g	2.25 (0.02) a-i	38	OSR10	4.17 (0.17) c-g	0.72 (0.22) h-j
14	EPR7	4.95 (0.15) a-g	1.05 (0.05) e-j	39	IKR11	6.04 (1.77) a-g	1.65 (0.25) a-j
15	UNS8	5.40 (0.45) a-g	0.70 (0.27) h-j	40	SKR2	6.45 (1.35) a-g	1.95 (0.59) a-j
16	AT-SKR	6.62 (0.12) a-g	2.40 (0.13) a-i	41	SKR5	4.72 (0.49) a-g	1.65 (0.25) a-j
17	IBS8	6.97 (0.04) a-f	2.97 (0.04) a-c	42	IGGR8	4.52 (0.25) a-g	0.92 (0.09) f-j
18	AT-IKS	8.09 (0.19) a	2.13 (010) a-j	43	IPR5	5.22 (0.25) a-g	1.18 (0.05) c-j
19	ILS13	5.52 (1.59) a-g	2.17 (0.07) a-j	44	TDR6	5.60 (0.70) a-g	1.45 (0.02) a-j
20	ILR6	6.34 (0.21) a-g	2.67 (0.14) a-g	45	TDS9	6.38 (0.48) a-g	2.19 (0.82) a-j
21	EPR1	4.82 (0.02) a-g	1.30 (0.20) b-j	46	UNR3	5.65 (0.48) a-g	1.37 (0.10) b-j
22	IPR1	6.82 (0.65) a-g	1.98 (0.08) a-j	47	OSR7	5.15 (0.85) a-g	1.83 (0.33) a-j
23	IGR1	3.60 (0.24) e-g	0.87 (0.14) f-j	48	UNS9	4.07 (0.07) d-g	0.37 (0.17) j
24	AJS2	4.54 (0.07) a-g	1.02 (0.25) f-j		LSD ($\alpha = 0.05$)	3.61	1.85

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences.

Table 4.12: Antagonist-Pathogen interaction effect on maize seed germination in the screen house

		Germination (%)				Germination (%)	
S/N	Treatments	Control	Ant + Path	S/N	Treatments	Control	Ant + Path
0	MZ (-VE)	90.00 (0.00) ab	80.00 (0.00) c-f	24	AJS2	96.67 (3.330 ab	90.00 (0.00) a-d
0	MZ + PT (+VE)	73.33 (8.82) c	63.33 (3.33) g	25	AKR2	100.00 (0.00) a	100.00 (0.00) a
1	AKR5	100.00 (0.00) a	80.00 (0.00) c-f	26	IWS1	100.00 (0.00) a	90.00 (0.00) a-d
2	AT-ILR	90.00 (0.00) ab	90.00 (0.00) a-d	27	EBR1	100.00 (0.00) a	100.00 (0.00) a
3	IGBR11	100.00 (0.00) a	93.33 (3.33) a-c	28	SKS3	100.00 (0.00) a	100.00 (0.00) a
4	IPR2	96.67 (3.33) ab	90.00 (0.00) a-d	29	ADS14	90.00 (0.00) ab	90.00 (0.00) a-d
5	AKS2	100.00 (0.00) a	100.00 (0.00) a	30	IBR6	96.67 (3.33) ab	90.00 (0.00) a-d
6	EPR3	90.00 (0.00) ab	90.00 (0.00) a-d	31	ABS6	100.00 (0.00) a	80.00 (0.00) c-f
7	AKR8	100.00 (0.00) a	100.00 (0.00) a	32	IGGR11	90.00 (0.00) ab	90.00 (0.00) a-d
8	EPR4	96.67 (3.33) ab	93.33 (3.33) a-c	33	IKR1	93.33 (3.33) ab	80.00 (0.00) c-f
9	AT-EPS	100.00 (0.00) a	90.00 (0.00) a-d	34	ILS14	90.00 (5.77) ab	80.00 (0.00) c-f
10	EBR4	100.00 (0.00) a	80.00 (0.00) c-f	35	IGGR5	86.67 (6.67) ab	80.00 (0.00) c-f
11	EPR2	90.00 (0.00) ab	76.67 (3.33) d-f	36	IWR2	93.33 (3.33) ab	86.67 (3.33) a-d
12	EBS8	90.00 (0.00) ab	90.00 (0.00) a-d	37	ABS8	100.00 (0.00) a	100.00 (0.00) a
13	SPS1	93.33 (3.33) ab	83.33 (3.33) b-e	38	OSR10	93.33 (3.33) ab	90.00 (0.00) a-d
14	EPR7	100.00 (0.00) a	100.00 (0.00) a	39	IKR11	100.00 (0.00) a	93.33 (3.33) a-c
15	UNS8	93.33 (6.67) ab	66.67 (6.67) fg	40	SKR2	93.33 (6.67) ab	80.00 (0.00) c-f
16	AT-SKR	100.00 (0.00) a	83.33 (6.67) b-e	41	SKR5	90.00 (0.00) ab	83.33 (3.33) b-e
17	IBS8	96.67 (3.33) ab	90.00 (0.00) a-d	42	IGGR8	90.00 (5.77) ab	80.00 (0.00) c-f
18	AT-IKS	100.00 (0.00) a	90.00 (5.77) a-d	43	IPR5	80.00 (0.00) bc	70.00 (10.00) e-g
19	ILS13	100.00 (0.00) a	76.67 (6.67) d-f	44	TDR6	100.00 (0.00) a	100.00 (0.00) a
20	ILR6	96.67 (3.33) ab	70.00 (0.00) e-g	45	TDS9	96.67 (3.33) ab	96.67 (3.33) ab
21	EPR1	100.00 (0.00) a	80.00 (0.00) c-f	46	UNR3	86.67 (8.82) ab	70.00 (10.00) e-g
22	IPR1	93.33 (6.67) ab	80.00 (0.00) c-f	47	OSR7	100.00 (0.00) a	80.00 (0.00) c-f
23	IGR1	100.00 (0.00) a	80.00 (0.00) c-f	48	UNS9	96.67 (3.33) ab	90.00 (0.00) a-d
					LSD ($\alpha = 0.05$)	9.43	8.17

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. Ant = Antagonist, Path = Pathogen.

Table 4.13: Disease expression during antagonist-pathogen interaction in the screenhouse

		Disease expression (%)				Disease expression (%)	
S/N	Treatments	Day 14	Day 21	S/N	Treatments	Day 14	Day 21
0	MZ (-VE)	0.00 (0.00) f	0.00 (0.00) g	24	AJS2	0.00 (0.00) f	11.10 (0.00) fg
0	MZ + PT (+VE)	88.00 (1.00) a	79.00 (4.00) a	25	AKR2	0.00 (0.00) f	0.00 (0.00) g
1	AKR5	0.00 (0.00) f	25.00 (0.00) e	26	IWS1	0.00 (0.00) f	11.10 (0.00) fg
2	AT-ILR	11.10 (0.00) e	11.10 (0.00) fg	27	EBR1	0.00 (0.00) f	0.00 (0.00) g
3	IGBR11	0.00 (0.00) f	11.10 (0.00) fg	28	SKS3	0.00 (0.00) f	0.00 (0.00) g
4	IPR2	0.00 (0.00) f	11.10 (0.00) fg	29	ADS14	11.10 (0.00) e	11.10 (0.00) fg
5	AKS2	0.00 (0.00) f	0.00 (0.00) g	30	IBR6	0.00 (0.00) f	11.10 (0.00) fg
6	EPR3	11.10 (0.00) e	11.10 (0.00) fg	31	ABS6	0.00 (0.00) f	25.00 (0.00) e
7	AKR8	0.00 (0.00) f	0.00 (0.00) g	32	IGGR11	11.10 (0.00) e	11.10 (0.00) fg
8	EPR4	0.00 (0.00) f	10.73 (0.37) g	33	IKR1	12.50 (0.00) e	25.00 (0.00) e
9	AT-EPS	0.00 (0.00) f	11.10 (0.00) fg	34	ILS14	0.00 (0.00) f	25.00 (0.00) e
10	EBR4	0.00 (0.00) f	25.00 (0.00) e	35	IGGR5	25.00 (0.00) d	25.00 (0.00) e
11	EPR2	26.47 (2.13) d	52.37 (2.37) c	36	IWR2	11.57 (0.47) e	11.57 (0.47) fg
12	EBS8	11.10 (0.00) e	22.40 (0.00) ef	37	ABS8	0.00 (0.00) f	0.00 (0.00) g
13	SPS1	0.00 (0.00) f	12.03 (0.47) fg	38	OSR10	0.00 (0.00) f	11.10 (0.00) fg
14	EPR7	0.00 (0.00) f	0.00 (0.00) g	39	IKR11	0.00 (0.00) f	0.00 (0.00) g
15	UNS8	30.53 (2.77) c	61.13 (5.57) b	40	SKR2	25.00 (0.00) d	25.00 (0.00) e
16	AT-SKR	0.00 (0.00) f	36.00 (3.20) d	41	SKR5	12.03 (0.47) e	24.07 (0.93) e
17	IBS8	0.00 (0.00) f	11.10 (0.00) fg	42	IGGR8	12.50 (0.00) e	25.00 (0.00) e
18	AT-IKS	0.00 (0.00) f	22.40 (1.45) ef	43	IPR5	30.00 (5.00) c	75.00 (2.50) a
19	ILS13	0.00 (0.00) f	39.70 (3.20) d	44	TDR6	0.00 (0.00) f	0.00 (0.00) g
20	ILR6	0.00 (0.00) f	42.90 (0.00) d	45	TDS9	0.00 (0.00) f	0.00 (0.00) g
21	EPR1	0.00 (0.00) f	25.00 (0.00) e	46	UNR3	42.9 (0.00) b	42.90 (0.00) d
22	IPR1	0.00 (0.00) f	25.00 (0.00) e	47	OSR7	12.70 (1.60) e	38.10 (0.00) d
23	IGR1	0.00 (0.00) f	25.00 (0.00) e	48	UNS9	0.00 (0.00) f	11.10 (0.00) fg
					LSD ($\alpha = 0.05$)	2.57	6.34

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences.

Table 4.14: Antagonist-Pathogen interaction effects on maize plant height at day 21.

		Plant height (cm)				Plant height (cm)	
S/N	Treatments	Control	Ant + Path	S/N	Treatments	Control	Ant + Path
0	MZ (-VE)	19.70 (0.70) ab	19.00 (1.00) a-c	24	AJS2	18.43 (0.42) ab	20.00 (0.53) a-c
0	MZ + PT (+VE)	0.00 (0.00) c	0.00 (0.00) d	25	AKR2	19.63 (0.57) ab	20.00 (0.76) a-c
1	AKR5	18.63 (1.32) ab	21.57 (2.51) a-c	26	IWS1	18.03 (0.29) ab	19.10 (0.76) a-c
2	AT-ILR	19.07 (0.55) ab	18.17 (1.20) a-c	27	EBR1	20.27 (1.08) ab	18.90 (0.38) a-c
3	IGBR11	19.63 (0.35) ab	19.10 (0.06) a-c	28	SKS3	18.67 (0.30) ab	18.17 (0.09) a-c
4	IPR2	18.43 (0.49) ab	21.30 (0.65) a-c	29	ADS14	19.53 (1.03) ab	19.70 (0.55) a-c
5	AKS2	18.57 (0.58) ab	20.20 (1.35) a-c	30	IBR6	19.47 (1.19) ab	21.00 (0.76) a-c
6	EPR3	17.67 (0.07) ab	21.17 (0.09) a-c	31	ABS6	20.10 (0.80) ab	19.13 (0.07) a-c
7	AKR8	19.67 (0.68) ab	20.93 (0.47) a-c	32	IGGR11	18.53 (1.31) ab	18.33 (0.12) a-c
8	EPR4	20.83 (0.62) ab	20.33 (0.67) a-c	33	IKR1	20.33 (0.20) ab	20.33 (1.17) a-c
9	AT-EPS	18.27 (0.65) ab	20.90 (0.98) a-c	34	ILS14	20.40 (0.46) ab	19.50 (0.77) a-c
10	EBR4	18.93 (0.09) ab	18.53 (0.39) a-c	35	IGGR5	20.97 (0.53) ab	19.73 (0.23) a-c
11	EPR2	18.87 (0.47) ab	18.20 (0.20) a-c	36	IWR2	20.00 (0.35) ab	21.50 (0.25) a-c
12	EBS8	20.00 (1.53) ab	20.00 (0.58) a-c	37	ABS8	13.73 (6.52) b	20.20 (0.31) a-c
13	SPS1	20.30 (0.70) ab	16.83 (0.38) bc	38	OSR10	20.93 (0.76) ab	20.03 (0.79) a-c
14	EPR7	17.47 (0.84) ab	16.87 (0.63) bc	39	IKR11	22.30 (1.65) a	21.53 (1.76) a-c
15	UNS8	19.93 (1.92) ab	19.47 (1.40) a-c	40	SKR2	20.27 (0.93) ab	18.60 (0.38) a-c
16	AT-SKR	18.30 (0.95) ab	21.83 (1.18) ab	41	SKR5	18.97 (1.48) ab	20.37 (0.70) a-c
17	IBS8	19.03 (0.91) ab	20.70 (0.90) a-c	42	IGGR8	18.50 (1.03) ab	19.60 (0.74) a-c
18	AT-IKS	18.77 (1.23) ab	19.60 (0.31) a-c	43	IPR5	19.07 (1.39) ab	19.83 (0.60) a-c
19	ILS13	19.30 (0.25) ab	21.93 (1.10) ab	44	TDR6	18.33 (0.72) ab	22.20 (1.40) a
20	ILR6	20.70 (0.72) ab	16.50 (0.76) c	45	TDS9	19.93 (1.37) ab	19.17 (0.17) a-c
21	EPR1	19.27 (1.07) ab	20.50 (1.26) a-c	46	UNR3	19.27 (0.12) ab	20.10 (0.90) a-c
22	IPR1	19.77 (0.70) ab	20.63 (0.53) a-c	47	OSR7	17.45 (0.05) ab	21.00 (0.00) a-c
23	IGR1	19.80 (0.15) ab	20.83 (0.73) a-c	48	UNS9	18.57 (0.75) ab	19.83 (0.82) a-c
					LSD ($\alpha = 0.05$)	3.65	2.49

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. Ant = Antagonist, Path = Pathogen.

Table 4.15: Antagonist-Pathogen interaction effect on maize stem girth at day 21

		Stem girth (cm)				Stem girth (cm)	
S/N	Treatments	Control	Ant + Path	S/N	Treatments	Control	Ant + Path
0	MZ (-VE)	0.51 (0.07) ab	0.57 (0.11) a	24	AJS2	0.47 (0.02) a-c	0.30 (0.00) b
0	MZ + PT (+VE)	0.00 (0.00) d	0.00 (0.00) c	25	AKR2	0.34 (0.05) bc	0.40 (0.00) ab
1	AKR5	0.35 (0.06) bc	0.37 (0.03) ab	26	IWS1	0.30 (0.02) bc	0.43 (0.03) ab
2	AT-ILR	0.31 (0.03) bc	0.37 (0.03) ab	27	EBR1	0.30 (0.03) bc	0.30 (0.00) b
3	IGBR11	0.30 (0.03) bc	0.37 (0.03) ab	28	SKS3	0.32 (0.02) bc	0.37 (0.03) ab
4	IPR2	0.38 (0.03) bc	0.42 (0.00) ab	29	ADS14	0.34 (0.02) bc	0.37 (0.03) ab
5	AKS2	0.40 (0.01) bc	0.33 (0.03) ab	30	IBR6	0.29 (0.07) bc	0.37 (0.03) ab
6	EPR3	0.34 (0.05) bc	0.40 (0.00) ab	31	ABS6	0.35 (0.06) bc	0.33 (0.03) ab
7	AKR8	0.44 (0.07) a-c	0.33 (0.03) ab	32	IGGR11	0.31 (0.04) bc	0.30 (0.00) b
8	EPR4	0.36 (0.05) bc	0.39 (0.02) ab	33	IKR1	0.36 (0.02) bc	0.40 (0.00) ab
9	AT-EPS	0.37 (0.02) bc	0.37 (0.03) ab	34	ILS14	0.33 (0.02) bc	0.43 (0.03) ab
10	EBR4	0.36 (0.01) bc	0.33 (0.03) ab	35	IGGR5	0.39 (0.03) bc	0.37 (0.07) ab
11	EPR2	0.47 (0.05) a-c	0.36 (0.07) ab	36	IWR2	0.34 (0.01) bc	0.33 (0.03) ab
12	EBS8	0.40 (0.03) bc	0.46 (0.08) ab	37	ABS8	0.32 (0.05) bc	0.37 (0.03) ab
13	SPS1	0.31 (0.04) bc	0.33 (0.03) ab	38	OSR10	0.31 (0.07) bc	0.43 (0.07) ab
14	EPR7	0.29 (0.03) bc	0.31 (0.01) b	39	IKR11	0.33 (0.03) bc	0.37 (0.07) ab
15	UNS8	0.22 (0.12) c	0.37 (0.03) ab	40	SKR2	0.31 (0.04) bc	0.30 (0.00) b
16	AT-SKR	0.25 (0.01) bc	0.33 (0.03) ab	41	SKR5	0.33 (0.05) bc	0.30 (0.00) b
17	IBS8	0.28 (0.04) bc	0.37 (0.03) ab	42	IGGR8	0.34 (0.07) bc	0.40 (0.10) ab
18	AT-IKS	0.27 (0.02) bc	0.40 (0.00) ab	43	IPR5	0.35 (0.07) bc	0.44 (0.06) ab
19	ILS13	0.29 (0.04) bc	0.40 (0.06) ab	44	TDR6	0.39 (0.02) bc	0.37 (0.03) ab
20	ILR6	0.22 (0.04) c	0.33 (0.03) ab	45	TDS9	0.38 (0.04) bc	0.30 (0.00) b
21	EPR1	0.34 (0.09) bc	0.40 (0.06) ab	46	UNR3	0.28 (0.03) bc	0.37 (0.03) ab
22	IPR1	0.26 (0.00) bc	0.40 (0.06) ab	47	OSR7	0.40 (0.00) bc	0.50 (0.05) ab
23	IGR1	0.24 (0.05) bc	0.40 (0.06) ab	48	UNS9	0.34 (0.09) bc	0.30 (0.00) b
					LSD ($\alpha = 0.05$)	0.13	0.12

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. Ant = Antagonist, Path = Pathogen.

Table 4.16: Antagonist-Pathogen interaction effect on maize plant number of leaves at day 21

		Number of leaves				Number of leaves	
S/N	Treatments	Control	Ant + Path	S/N	Treatments	Control	Ant + Path
0	MZ (-VE)	6.30 (0.33) a	6.33 (0.33) a	24	AJS2	6.67 (0.33) a	6.67 (0.33) a
0	MZ + PT (+VE)	0.00 (0.00) b	0.00 (0.00) b	25	AKR2	6.33 (0.33) a	6.67 (0.33) a
1	AKR5	6.67 (0.33) a	6.67 (0.33) a	26	IWS1	6.67 (0.33) a	7.00 (0.00) a
2	AT-ILR	6.67 (0.33) a	6.33 (0.33) a	27	EBR1	6.67 (0.33) a	7.00 (0.00) a
3	IGBR11	7.00 (0.00) a	7.00 (0.00) a	28	SKS3	6.67 (0.33) a	6.33 (0.33) a
4	IPR2	6.33 (0.33) a	6.67 (0.33) a	29	ADS14	6.67 (0.33) a	6.33 (0.55) a
5	AKS2	6.67 (0.33) a	7.00 (0.00) a	30	IBR6	6.00 (0.00) a	7.00 (0.58) a
6	EPR3	6.67 (0.33) a	7.33 (0.67) a	31	ABS6	6.00 (0.00) a	6.67 (0.33) a
7	AKR8	7.00 (0.00) a	6.00 (0.56) a	32	IGGR11	7.00 (0.00) a	6.00 (0.00) a
8	EPR4	6.33 (0.33) a	6.33 (0.33) a	33	IKR1	6.67 (0.33) a	6.67 (0.33) a
9	AT-EPS	7.00 (0.00) a	6.00 (0.33) a	34	ILS14	6.33 (0.33) a	6.33 (0.33) a
10	EBR4	6.67 (0.33) a	7.00 (0.00) a	35	IGGR5	7.00 (0.58) a	7.00 (0.00) a
11	EPR2	6.33 (0.33) a	5.33 (0.33) a	36	IWR2	6.00 (0.00) a	6.33 (0.67) a
12	EBS8	7.00 (0.00) a	6.00 (0.00) a	37	ABS8	6.67 (0.33) a	6.67 (0.33) a
13	SPS1	6.67 (0.33) a	7.00 (0.00) a	38	OSR10	6.00 (0.00) a	6.67 (0.33) a
14	EPR7	6.00 (0.00) a	7.00 (0.58) a	39	IKR11	6.00 (0.00) a	7.00 (0.58) a
15	UNS8	6.33 (0.33) a	6.67 (0.33) a	40	SKR2	6.33 (0.33) a	7.33 (0.88) a
16	AT-SKR	7.00 (0.00) a	6.00 (0.00) a	41	SKR5	6.00 (0.00) a	6.67 (0.33) a
17	IBS8	6.00 (0.00) a	6.67 (0.33) a	42	IGGR8	6.33 (0.33) a	6.67 (0.33) a
18	AT-IKS	6.67 (0.67) a	6.67 (0.33) a	43	IPR5	6.67 (0.33) a	6.00 (0.00) a
19	ILS13	6.00 (0.00) a	6.67 (0.33) a	44	TDR6	6.33 (0.33) a	6.67 (0.33) a
20	ILR6	7.00 (0.58) a	6.67 (0.67) a	45	TDS9	6.67 (0.33) a	6.67 (0.33) a
21	EPR1	6.67 (0.33) a	6.67 (0.33) a	46	UNR3	5.67 (0.33) a	6.33 (0.33) a
22	IPR1	6.00 (0.58) a	6.67 (0.33) a	47	OSR7	5.50 (0.00) a	7.00 (0.00) a
23	IGR1	6.67 (0.33) a	6.00 (0.00) a	48	UNS9	7.00 (0.58) a	6.67 (0.33) a
					LSD ($\alpha = 0.05$)	0.91	1.06

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. Ant = Antagonist, Path = Pathogen.

Table 4.17: Antagonist-Pathogen interaction effect on maize leaf area at day 21

		Leaf area (cm^2)				Leaf area (cm^2)	
S/N	Treatments	Control	Ant + Path	S/N	Treatments	Control	Ant + Path
0	MZ (-VE)	84.30 (15.08) ab	80.60 (3.90) ab	24	AJS2	92.10 (8.61) ab	81.80 (10.49) ab
0	MZ + PT (+VE)	0.00 (0.00) c	71.70 (2.69) ab	25	AKR2	76.50 (4.54) ab	94.37 (13.70) ab
1	AKR5	87.33 (9.65) ab	110.23 (5.52) ab	26	IWS1	73.47 (10.82) ab	83.00 (10.03) ab
2	AT-ILR	63.63 (3.20) ab	93.20 (3.38) ab	27	EBR1	79.20 (11.66) ab	81.73 (4.26) ab
3	IGBR11	72.90 (3.46) ab	93.07 (3.59) ab	28	SKS3	75.10 (4.80) ab	71.17 (9.42) ab
4	IPR2	89.40 (15.12) ab	80.30 (4.76) ab	29	ADS14	70.73 (6.19) ab	79.67 (4.89) ab
5	AKS2	82.50 (8.26) ab	91.73 (1.13) ab	30	IBR6	77.30 (2.65) ab	97.47 (13.02) ab
6	EPR3	74.80 (8.16) ab	86.97 (4.79) ab	31	ABS6	88.50 (2.18) ab	72.97 (8.81) ab
7	AKR8	113.20 (4.68) a	60.93 (1.08) b	32	IGGR11	96.93 (6.63) ab	88.90 (13.66) ab
8	EPR4	84.27 (9.08) ab	88.20 (11.75) ab	33	IKR1	91.30 (0.90) ab	93.87 (2.98) ab
9	AT-EPS	94.37 (8.89) ab	89.83 (7.67) ab	34	ILS14	79.17 (8.76) ab	76.97 (0.23) ab
10	EBR4	67.27 (7.89) ab	84.03 (4.87) ab	35	IGGR5	92.53 (0.98) ab	90.20 (6.38) ab
11	EPR2	88.53 (8.80) ab	71.70 (2.69) ab	36	IWR2	68.33 (2.28) ab	77.80 (6.08) ab
12	EBS8	105.67 (3.44) ab	75.73 (9.33) ab	37	ABS8	98.57 (5.06) ab	85.23 (13.76) ab
13	SPS1	67.40 (15.93) ab	90.90 (10.20) ab	38	OSR10	85.90 (4.10) ab	75.07 (7.49) ab
14	EPR7	80.10 (8.44) ab	91.80 (4.50) ab	39	IKR11	71.55 (11.18) ab	84.00 (6.92) ab
15	UNS8	80.40 (11.8) ab	72.97 (8.80) ab	40	SKR2	90.83 (15.01) ab	115.07 (7.98) ab
16	AT-SKR	79.90 (4.45) ab	81.87 (5.90) ab	41	SKR5	75.10 (4.80) ab	75.67 (16.03) ab
17	IBS8	79.63 (7.19) ab	85.57 (3.87) ab	42	IGGR8	93.53 (15.08) ab	102.97 (10.92) ab
18	AT-IKS	72.90 (6.30) ab	63.33 (28.21) ab	43	IPR5	84.70 (12.35) ab	76.87 (8.71) ab
19	ILS13	71.13 (1.86) ab	78.13 (11.59) ab	44	TDR6	76.47 (8.87) ab	98.80 (8.04) ab
20	ILR6	83.13 (3.52) ab	69.47 (2.13) ab	45	TDS9	87.27 (6.91) ab	73.73 (10.37) ab
21	EPR1	74.27 (19.35) ab	89.73 (9.36) ab	46	UNR3	70.20 (2.89) ab	60.97 (7.96) b
22	IPR1	69.43 (12.43) ab	123.83 (12.76) ab	47	OSR7	81.90 (2.75) ab	122.75 (14.95) a
23	IGR1	71.67 (7.13) ab	84.87 (7.31) ab	48	UNS9	55.03 (10.53) ab	81.60 (9.77) ab
					LSD ($\alpha = 0.05$)	25.40	26.62

Mean values of three replicates are shown. Mean values followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. Ant = Antagonist, Path = Pathogen.

Table 4.18: Antagonist-Pathogen interaction effect on maize rhizosphere bacteria load

S/N	Treatments	Control (10^{-6} CFU/g soil)	Ant + Path (10^{-6} CFU/g soil)	S/N	Treatments	Control (10^{-6} CFU/g soil)	Ant + Path (10^{-6} CFU/g soil)
0	MZ (-VE)	56.67 (8.11) f	23.67 (8.76) o-q	24	AJS2	139 (8.62) c-e	77.00 (9.07) h-o
0	MZ + PT (+VE)	3.00 (1.53) g	3.40 (0.00) q	25	AKR2	179.33 (16.18) a-e	78.33 (15.80) g-o
1	AKR5	165.33 (27.50) b-e	31.00 (2.52) o-q	26	IWS1	147.00 (15.31) c-e	116.33 (12.44) a-k
2	AT-ILR	246.33 (45.96) a-c	153.00 (7.00) a-d	27	EBR1	172.33 (6.33) a-e	87.33 (6.36) f-n
3	IGBR11	271.33 (33.95) a	114.67 (4.81) a-k	28	SKS3	167.00 (5.86) b-e	134.00 (6.43) a-i
4	IPR2	209.00 (25.11) a-e	139.33 (5.93) a-h	29	ADS14	197.33 (12.72) a-e	130.00 (22.30) a-i
5	AKS2	163.00 (15.31) b-e	151.67 (16.58) a-e	30	IBR6	175.00 (5.13) a-e	113.33 (8.11) a-k
6	EPR3	182.00 (10.26) a-e	168.00 (9.24) a	31	ABS6	184.67 (25.10) a-e	65.33 (1.33) j-p
7	AKR8	102.67 (5.81) d-f	21.00 (1.00) pq	32	IGGR11	160.00 (4.00) b-e	39.00 (3.61) n-q
8	EPR4	232.00 (8.33) a-c	165.00 (8.14) ab	33	IKR1	184.33 (34.57) a-e	89.00 (5.51) e-n
9	AT-EPS	192.00 (27.71) a-e	161.00 (17.32) ab	34	ILS14	185.67 (11.02) a-e	140.67 (10.48) a-g
10	EBR4	192.67 (8.67) a-e	163.00 (4.73) ab	35	IGGR5	187.67 (8.25) a-e	93.33 (14.85) d-n
11	EPR2	185.67 (20.92) a-e	62.67 (4.81) k-p	36	IWR2	181.00 (19.40) a-e	132.33 (8.09) a-i
12	EBS8	160.67 (20.54) b-e	78.00 (2.00) c-m	37	ABS8	108.33 (18.22) d-f	73.00 (7.00) i-p
13	SPS1	155.67 (6.91) b-e	92.33 (4.63) d-n	38	OSR10	180.33 (29.08) a-e	120.00 (23.44) a-k
14	EPR7	166.00 (25.32) b-e	117.00 (9.27) a-k	39	IKR11	172.00 (8.33) a-e	138.33 (13.22) a-h
15	UNS8	185.67 (13.96) a-e	43.00 (11.93) m-q	40	SKR2	260.00 (31.75) ab	75.00 (11.59) i-p
16	AT-SKR	207.67 (4.33) a-e	117.00 (10.69) a-k	41	SKR5	159.33 (18.77) b-e	78.33 (5.78) g-o
17	IBS8	187.33 (22.58) a-e	158.67 (17.64) a-c	42	IGGR8	178.33 (11.02) a-e	105.33 (15.38) a-l
18	AT-IKS	215.33 (15.65) a-d	125.67 (29.28) a-j	43	IPR5	212.00 (15.14) a-d	133.00 (23.29) a-i
19	ILS13	142.00 (6.43) c-e	90.67 (10.91) d-n	44	TDR6	159.00 (13.00) b-e	121.67 (3.84) a-k
20	ILR6	227.00 (15.95) a-c	142.67 (22.43) a-f	45	TDS9	181.33 (2.67) a-e	132.33 (7.22) a-k
21	EPR1	235.67 (22.15) a-c	123.67 (3.67) a-k	46	UNR3	151.00 (21.8) c-e	108.00 (6.11) a-k
22	IPR1	202.33 (19.32) a-e	141.67 (4.48) a-g	47	OSR7	188.00 (6.00) a-e	48.00 (0.00) l-q
23	IGR1	164.00 (6.11) b-e	113.67 (5.04) a-k	48	UNS9	187.67 (8.65) a-e	102.33 (4.48) b-l
					LSD ($\alpha = 0.05$)	52.89	32.65

Mean values of three replicates followed by the same letter in the columns indicate significant differences according to Student-Newman-Keuls multiple-range test ($\alpha = 0.05$). LSD = Least Significant Differences. CFU = Colony Forming Unit. Ant = Antagonist, Path = Pathogen

Table 4.19: Plant growth promoting characteristics of bacteria isolates effectiveness (%)

Evaluation and screening parameters	Effectiveness (%)
Phosphate solubilization	61.20
Potentials to produce chitinase (In-vitro)	63.30
Indole -3-acetic acid (L-Trytophan)	100.00
Indole -3-acetic acid (D-L-Trytophan)	100.00
Maize seed germination (Laboratory)	100.00
Radicle length	60.42
Plumule length	47.20
Maize seed germination (Screen house)	100.00
Disease expression at day 14	12.24
Disease expression at day 21	43.75
Plant height	81.25
Stem girth	0.00
Leaf number	83.33
Leaf area	68.75
Microbial load	97.92

Evaluation and screening parameters based on plant growth promoting characteristics of bacteria isolates are compared with the controls to determine their percentage effectiveness.

Table 4.20: Pearson correlation coefficients on agronomical and pathological data obtained from maize treated with 48 bacteria isolates.

	GERM (LB)	RL	PL	GERM (SH)	DE 14D	DE 21D	PH	SG	LN	LA
RL	0.10 ^{ns}									
PL	0.08^{ns}	0.73***								
GERM (SH)	-0.07 ^{ns}	-0.05 ^{ns}	-0.05 ^{ns}							
DE 14D	-0.02 ^{ns}	0.06 ^{ns}	-0.05 ^{ns}	-0.55***						
DE 21D	0.06 ^{ns}	0.03 ^{ns}	-0.01 ^{ns}	-0.88***	0.64***					
PH	-0.05 ^{ns}	-0.07 ^{ns}	-0.08 ^{ns}	0.06 ^{ns}	-0.07 ^{ns}	-0.04 ^{ns}				
SG	-0.01 ^{ns}	-0.10 ^{ns}	-0.05 ^{ns}	-0.15 ^{ns}	0.05 ^{ns}	0.17*	0.09 ^{ns}			
LN	-0.02 ^{ns}	0.01 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	-0.12 ^{ns}	-0.20*	-0.08 ^{ns}	-0.01 ^{ns}		
LA	0.02 ^{ns}	0.04 ^{ns}	-0.01 ^{ns}	-0.03 ^{ns}	-0.06 ^{ns}	-0.06 ^{ns}	-0.02 ^{ns}	-0.04 ^{ns}	0.10 ^{ns}	
BL	-0.20*	0.07 ^{ns}	0.03 ^{ns}	0.13 ^{ns}	-0.23**	-0.18*	-0.02 ^{ns}	0.12 ^{ns}	0.09 ^{ns}	0.00 ^{ns}

RL = Radicle length, PL = Plumule length, GERM (SH) = Germination in the screen house, DE 14D = Disease expression at day 14, DE 21D = Disease expression at day 21, PH = Plant height, SG = Stem girth, LN = Leaf number, LA = Leaf area, BL = Bacteria load.

*= Significant at P < 0.05
**= Significant at P < 0.01
***= Significant at P < 0.0001
ns = not significant at P < 0.05, P < 0.01, P < 0.000

Table 4.21: Preliminary identification of bacteria isolates

S / N	Isolate code	Suspected bacteria isolates
I	EPR2	*Bacillus cereus*
2	EBS8	*Bacillus subtilis*
3	EPR4	*Pseudomonas mallei*
4	ABS6	*Pseudomonas mallei*
5	EPR7	*Bacillus subtilis*
6	TDS9	*Azomonas insignis*
7	IGBR11	*Azomonas insignis*
8	ADS14	*Pseudomonas pseudomallei*
9	IBS8	*Pseudomonas mallei*
10	OSR7	*Azomonas insignis*
11	EPR3	*Xanthomonas fragariae*
12	AT-SKR	*Azomonas insignis*
13	IPR1	*Azomonas insignis*
14	ILS13	*Bacillus subtilis*
15	AT-IKS	*Pseudomonas mallei*
16	AKR5	*Xanthomonas ampelina*
17	AT-ILR	*Azomonas macrocytogenes*
18	IGGR11	*Pseudomonas mallei*
19	UNS9	*Pseudomonas alcaligenes*

Table 4.22: Distribution of beneficial bacteria isolates based on ecological zone

Bacteria Isolates	GS		DS		LR		FW		MF		R (%)	S (%)
	R	S	R	S	R	S	R	S	R	S	R	S
EPR2							+	-			1	0
EBS8									-	+	0	1
EPR4							+	-			1	0
ABS6							-	+			0	1
EPR7							+	-			1	0
TDS9	-	+									0	1
IGBR11							+	-			1	0
ADS14							-	+			0	1
IBS8					-	+					0	1
OSR7					+	-					1	0
EPR3							+	-			1	0
AT-SKR	+	-									1	0
IPR1					+	-					1	0
ILS13			-	+							0	1
AT-IKS			-	+							0	1
AKR5									+	-	1	0
AT-ILR			+	-							1	0
IGGR11			+	-							1	0
UNS9									-	+	0	1
Total	1	1	2	2	2	1	5	2	1	2	**11**	**8**
Grand total	2		4		3		7		3		**19**	

Guinea Savannah (GS), Derived Savannah (DS), Lowland Rainforest (LR), Fresh water forest (FW), Mangrove Forest (MF). R = Rhizosphere, S = Soil, % = Percentage. (+) Presence, (-) Absence.

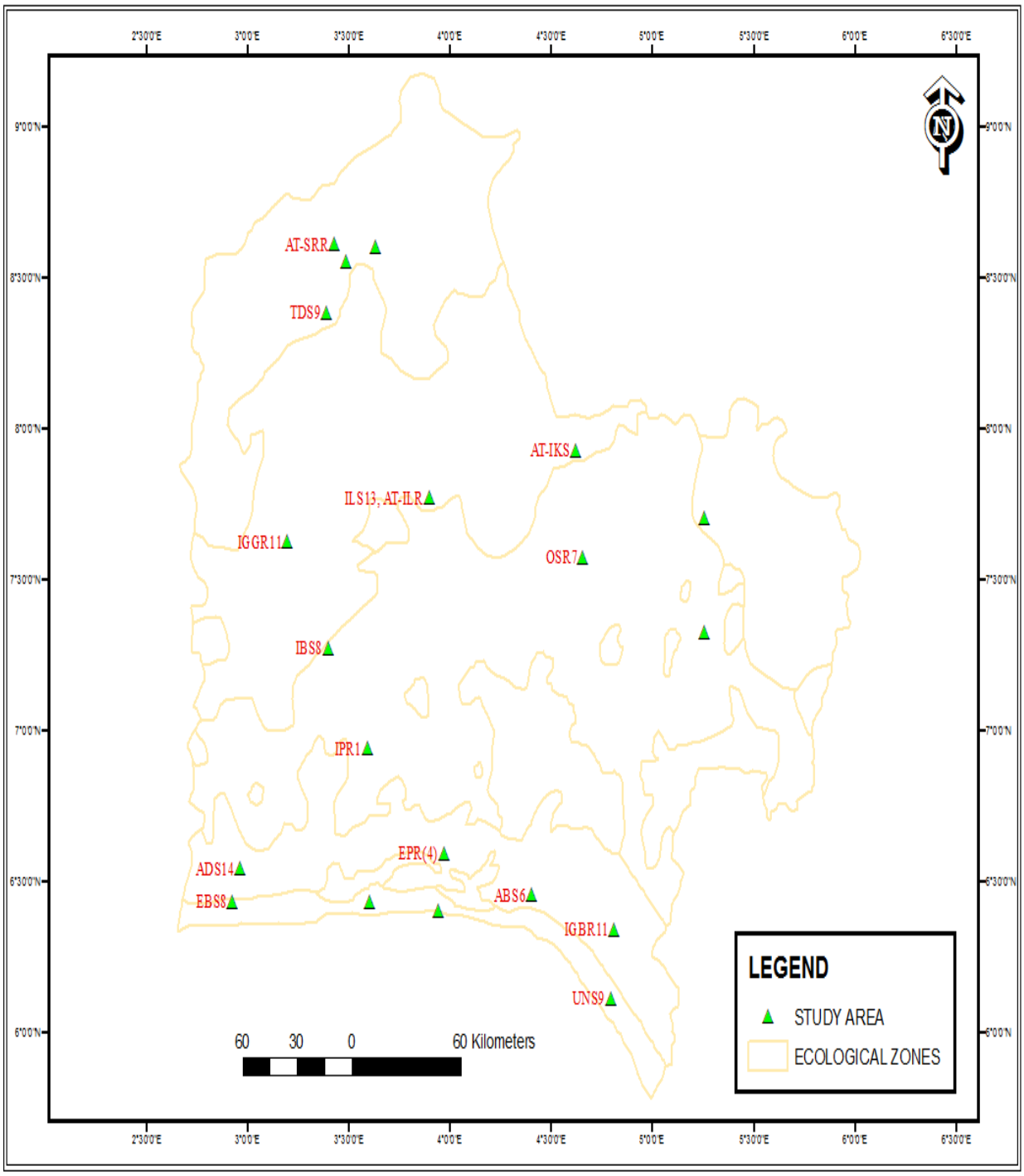


Figure 4.5: Map of Southwestern Nigeria showing distribution of beneficial bacteria isolates in ecological zones of southwestern Nigeria.

evaluated and analyzed so as to screen for the useful isolates. Nineteen (19) out of fourty eigth (48) isolates were observed to show potentials as plant growth promoter. However, further studies were carried out to justify the benefical effect of bacteria isolates on maize growth (Table 4.19 and Table 4.21).

4.8 Relationship between agronomical and pathological data from antagonist-pathogen interaction.

Significant ($P < 0.05$) positive and negative correlations were observed between the agronomical and pathological activities across treatments with correlation co-efficient ranging from 0.01 to 0.88 (Table 4.20). Radicle length and plumule length were observed with strong positive correlation ($r = 0.73$; $P < 0.0001$). Disease expression at day 14 was also observed to be correrlaed ($r = 0.64$; $P < 0.0001$) to disease expression at day 21. Bacterial load was negatively correlated to disease expression at day 14 ($r = -0.23$, $P < 0.01$) and day 21 ($r = -0.18$, $P < 0.05$). Strong negative correlation was observed between maize seed germination percentage in the screenhouse and disease expression at day 14 ($r = -0.55$, $P < 0.01$) and at day 21 ($r = -0.88$, $P < 0.0001$). Leaf number ($r = -0.20$, $P < 0.05$) and bacteria load ($r = -0.18$, $P < 0.01$) were significantly negatively correlated to disease expression at day 21. Also, the bacteria load ($r = -0.23$, $P < 0.01$) was negatively correlated to disease expression at day 14 (Table 4.20). Other variables were not significant.

4.9 Preliminary identification of bacteria isolates

Exactly 19 bacteria isolates showed significant ($P < 0.05$) plant growth promoting characteristics compared to others. The preliminary identification of bacteria isolates based on biochemical characterization, identified and suspected bacteria isolates as EPR2 = *Bacillus cereus,* EBS8 = *Bacillus subtilis,* EPR4 = *Pseudomonas mallei,* ABS6 = *Pseudomonas mallei,* EPR7 = *Azomonas insignis,* TDS9 = *Azomonas insignis,* IGBR11 = *Azomonas insignis,* ADS14 = *Pseudomonas pseudomallei,* IBS8 = *Bacillus subtilis,* OSR7 = *Azomonas insignis,* EPR3 = *Xanthomonas fragariae,* AT-SKR = *Azomonas insignis,* IPR1 = *Bacillus subtilis,* ILS13 = *Pseudomonas mallei,* AT-IKS = *Pseudomonas mallei,* AKR5 = *Xanthomonas ampelina,* AT-ILR = *Azomonas macrocytogenes,* IGGR11 = *Pseudomonas mallei,* UNS9 = *Pseudomonas alcaligenes* (Table 4.21). Suspected bacteria isolates identity were further confirmed using 16S rDNA molecular techniques.

Out of the 19 beneficial bacteria isolates, 11 were from the rhizosphere of maize, while the remaining 8 were from the counterpart soil. As regards each of the ecological zone, the beneficial bacteria were mostly from the rhizosphere of maize plant compared to the soil (Table 4.22). Virtually, each of the study area had one or more beneficial bacteria, only with the exception of Igboho, Iworoko, Akure, Aja and Akodo. Interestingly, Epe study area in Freshwater ecological zone had 4 (EPR2, EPR3, EPR4 and EPR7) beneficial bacteria (Figure 4.5).

4.9.1 Effect of beneficial bacteria inoculation on maize plant height: At 2 weeks after planting (2WAP), the positive control (MZ + NPK) treated maize had the shortest height (8.50 cm) and OSR7 treated maize plant had the highest height (14.30 cm) which was about 5% higher in height than the mean height of the maize plant across the treatments. On the contrary for 2WAP with respect to unsterilized soil, maize plant with treatment ADS14 had the shortest (7.63 cm) even shorter than the positive control (MZ + NPK) and the negative control (MZ alone) treated maize plant height. However, maize plant treated with EPR2 and EBS8 had the highest (14.07cm) height, though; they were observed not to be significantly different from each other (Table 4.23). At 4WAP, maize plant treated with NPK fertilizer (positive control) had the shortest (19.8 cm) height in sterilized soil and the highest (28.60 cm) in an unsterilized soil, though; TDS9 treatment enhanced maize height than other treatnents in sterilized soil. There were no significant differences in maize height across the treatments at harvest (6WAP) in both sterilized and unsterilized soil. UNS9 treatment had the highest (53.27 cm) height that was far better than other treatments including the controls in sterilized soil, while the maize plant treated with NPK fertilizer (positive control) had the highest (52.00 cm) in an unsterilized soil. Generally, the mean value of maize height justified that maize plant height were generally enhanced by the treatments in both sterilized and unsterilized soils (Table 4.23).

4.9.2 Effects of beneficial bacteria inoculation on maize stem girth: Maize stem girth does not show any significant differences across the treatments at 2WAP in both sterilized and unsterilized soils. However, stem girth of maize treated with OSR7, ADS14 and AKR5 were higher than the controls in sterilized soil while stem girth of maize treated with AKR5 was higher

Table 4.23: Effect of bacteria inoculation on plant height of maize in both sterilized and unsterilized soil

Weeks	**2WAP**		**4WAP**		**6WAP**	
Treatment	ST	UNST	ST	UNST	ST	UNST
Mz (-ve)	11.83 (0.54) a	12.30 (0.61) ab	23.80 (0.25) a	23.00 (0.00) ab	41.33 (0.81) a	39.53 (1.13) a
Mz + NPK (+ve)	**8.50 (1.23) a**	10.30 (0.49) ab	**19.83 (2.84) a**	**28.60 (2.23) a**	36.67 (4.15) a	**52.00 (0.93) a**
EPR2	13.80 (0.61) a	**14.07 (0.83) a**	29.43 (1.43) a	24.90 (0.26) ab	49.57 (6.39) a	43.67 (3.39) a
EBS8	14.93 (0.20) a	**14.07 (0.83) a**	29.43 (1.43) a	22.00 (1.15) ab	48.60 (2.71) a	38.20 (3.43) a
EPR4	13.78 (0.59) a	13.80 (0.65) ab	27.93 (1.11) a	26.73 (0.37) ab	51.83 (0.37) a	45.00 (0.87) a
ABS6	14.37 (1.35) a	12.33 (0.50) ab	24.20 (2.12) a	26.73 (0.82) ab	48.20 (7.65) a	46.67 (2.57) a
EPR7	12.97 (0.23) a	13.10 (0.45) ab	27.97 (1.82) a	21.70 (1.16) ab	51.53 (6.21) a	33.97 (0.98) a
TDS9	13.57 (0.84) a	13.80 (1.46) ab	**30.37 (1.94) a**	23.23 (2.04) ab	47.43 (2.69) a	38.97 (3.61) a
IGBR11	9.83 (4.93) a	10.50 (0.95) ab	20.43 (10.30) a	22.43 (1.27) ab	33.61 (16.83) a	38.97 (1.52) a
ADS14	12.47 (1.44) a	**7.63 (3.86) b**	27.67 (1.20) a	16.00 (3.02) b	50.27 (2.03) a	31.30 (15.65) a
IBS8	14.10 (0.47) a	11.70 (0.32) ab	32.10 (2.05) a	23.97 (0.99) ab	59.57 (6.96) a	45.20 (0.95) a
OSR7	**14.30 (0.55) a**	13.33 (0.13) ab	30.00 (3.06) a	24.47 (0.75) ab	50.13 (5.16) a	46.10 (3.56) a
EPR3	14.10 (1.50) a	11.23 (0.75) ab	27.20 (2.82) a	23.67 (1.33) ab	51.13 (7.53) a	43.60 (0.70) a
AT-SKR	13.30 (0.90) a	12.07 (1.58) ab	24.10 (1.95) a	24.43 (1.82) ab	48.00 (4.24) a	47.20 (6.29) a
IPR1	13.67 (1.13) a	13.40 (0.36) ab	22.53 (0.68) a	22.93 (0.57) ab	48.33 (6.22) a	41.47 (3.03) a
ILS13	12.40 (0.42) a	12.70 (1.34) ab	25.30 (2.32) a	21.70 (4.11) ab	49.93 (5.49) a	31.23 (0.55) a
AT-IKS	14.60 (0.83) a	12.77 (0.52) ab	28.37 (1.24) a	25.27 (1.72) ab	56.63 (4.17) a	42.67 (4.19) a
AKR5	15.07 (0.59) a	12.10 (0.98) ab	28.70 (3.86) a	21.43 (0.87) ab	53.23 (6.67) a	42.93 (2.57) a
AT-ILR	12.80 (0.35) a	12.07 (1.25) ab	24.00 (1.00) a	24.20 (0.85) ab	46.50 (3.88) a	43.30 (1.80) a
IGGR11	12.20 (1.01) a	12.07 (1.25) ab	24.00 (1.00) a	24.90 (1.86) ab	46.23 (6.29) a	46.87 (2.94) a
UNS9	14.03 (0.09) a	12.03 (2.38) ab	29.10 (0.39) a	24.27 (1.13) ab	**53.27 (2.38) a**	46.90 (1.00) a
Mean	12.66	11.83	25.45	22.70	46.50	41.05
MSD ($\alpha = 0.05$)	7.30	6.27	15.65	11.15	32.21	23.04

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. WAP = Weeks After Planting, ST = Sterilized Soil, UNST= Unsterilized Soil.

Table 4.24: Effect of bacteria inoculation on stem girth of maize in both sterilized and unsterilized soil

Weeks	**2WAP**		**4WAP**		**6WAP**	
Treatment	ST	UNST	ST	UNST	ST	UNST
Mz (-ve)	0.75 (0.04) a	0.62 (0.07) cd	1.17 (0.06) a	1.24 (0.11) a	1.54 (0.09) a	1.78 (0.08) a
Mz + NPK (+ve)	0.56 (0.23) a	0.58 (0.07) d	1.30 (0.13) a	**2.00 (1.60) a**	**2.18 (0.41) a**	**2.38 (1.19) a**
EPR2	0.84 (0.10) a	0.82 (0.05) a-d	1.67 (0.08) a	1.37 (0.10) a	1.70 (0.03) a	1.79 (0.05) a
EBS8	0.67 (0.16) a	0.66 (0.03) b-d	1.63 (0.09) a	1.24 (0.07) a	1.77 (0.04) a	1.58 (0.01) a
EPR4	0.59 (0.05) a	0.92 (0.08) a-d	1.61 (0.13) a	1.48 (0.01) a	1.84 (0.05) a	1.58 (0.04) a
ABS6	0.82 (0.06) a	0.96 (0.05) a-d	1.24 (0.17) a	1.48 (0.07) a	1.59 (0.19) a	1.80 (0.10) a
EPR7	0.58 (0.03) a	0.86 (0.08) a-d	1.57 (0.14) a	1.16 (0.14) a	1.69 (0.26) a	1.63 (0.03) a
TDS9	0.71 (0.11) a	0.71 (0.01) a-d	1.15 (0.06) a	1.35 (0.150 a	1.81 (0.30) a	1.72 (0.11) a
IGBR11	0.70 (0.02) a	0.86 (0.03) a-d	1.08 (0.54) a	1.15 (0.10) a	1.16 (0.58) a	1.57 (0.14) a
ADS14	**0.85 (0.04) a**	0.84 (0.09) a-d	1.30 (0.08) a	0.91 (0.49) a	1.55 (0.06) a	1.06 (0.54) ab
IBS8	0.82 (0.08) a	0.99 (0.10) a-c	**1.77 (0.25) a**	1.37 (0.18) a	1.85 (0.25) a	1.65 (0.10) a
OSR7	**0.93 (0.03) a**	0.78 (0.03) a-d	1.65 (0.07) a	1.27 (0.19) a	1.91 (0.03) a	1.60 (0.09) a
EPR3	0.76 (0.03) a	0.69 (0.05) b-d	0.96 (0.31) a	1.33 (0.08) a	1.58 (0.13) a	1.82 (0.04) a
AT-SKR	0.79 (0.03) a	0.81 (0.05) a-d	1.23 (0.13) a	1.38 (0.09) a	1.61 (0.10) a	1.80 (0.12) a
IPR1	0.67 (0.03) a	1.04 (0.02) b	1.46 (0.02) a	1.25 (0.08) a	1.65 (0.05) a	1.48 (0.08) ab
ILS13	0.81 (0.14) a	0.72 (0.01) a-d	1.57 (0.09) a	1.35 (0.150 a	1.67 (0.13) a	1.65 (0.08) a
AT-IKS	0.64 (0.05) a	0.86 (0.03) a-d	1.50 (0.12) a	1.34 (0.12) a	1.77 (0.05) a	1.59 (0.13) a
AKR5	**0.85 (0.07) a**	**1.08 (0.04) a**	1.33 (0.12) a	1.57 (0.31) a	1.71 (0.15) a	1.61 (0.08) a
AT-ILR	0.49 (0.04) a	0.97 (0.17) a-c	1.37 (0.12) a	1.41 (0.19) a	1.89 (0.48) a	1.95 (0.17) a
IGGR11	0.54 (0.06) a	0.80 (0.02) a-d	1.49 (0.23) a	1.19 (0.08) a	1.51 (0.03) a	1.65 (0.04) a
UNS9	0.64 (0.01) a	0.95 (0.17) a-c	1.31 (0.09) a	1.31 (0.12) a	1.73 (0.11) a	1.79 (0.16) a
Mean	0.69	0.79	1.33	1.30	1.64	1.63
MSD ($\alpha = 0.05$)	0.46	0.39	0.94	1.44	1.21	1.56

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. WAP = Weeks After Planting, ST = Sterilized Soil, UNST= Unsterilized Soil.

Table 4.25: Effect of bacteria inoculation on number of leaves of maize in both sterilized and unsterilized soil

Weeks	**2WAP**		**4WAP**		**6WAP**	
Treatment	ST	UNST	ST	UNST	ST	UNST
Mz (-ve)	5.33 (0.33) a	5.00 (0.00) a	8.67 (0.33) a	8.33 (0.33) ab	11.00 (0.00) ab	10.33 (0.88) a
Mz + NPK (+ve)	5.00 (0.58) a	5.67 (0.33) a	8.00 (0.00) a	9.00 (0.00) a	11.33 (0.33) ab	12.33 (0.33) a
EPR2	5.67 (0.33) a	5.33 (0.33) a	8.67 (0.33) a	9.00 (0.00) a	12.33 (0.33) ab	11.33 (0.33) a
EBS8	5.00 (0.00) a	5.00 (0.00) a	9.00 (0.00) a	8.00 (0.00) ab	11.67 (0.33) ab	11.67 (0.00) a
EPR4	5.67 (0.33) a	5.67 (0.33) a	9.00 (0.00) a	8.67 (0.33) ab	12.00 (0.00) ab	11.33 (0.67) a
ABS6	5.67 (0.33) a	5.33 (0.67) a	8.67 (0.33) a	8.00 (0.58) ab	12.00 (0.58) ab	11.33 (0.33) a
EPR7	5.33 (0.33) a	3.33 (1.67) a	9.33 (0.33) a	5.23 (2.67) b	12.00 (0.00) ab	8.33 (4.18) a
TDS9	5.33 (0.33) a	4.67 (0.33) a	9.00 (0.58) a	8.33 (0.33) ab	11.33 (0.33) ab	12.33 (0.33) a
IGBR11	5.00 (0.00) a	5.33 (0.33) a	8.67 (0.33) a	8.00 (0.00) ab	8.00 (4.00) b	11.67 (0.00) a
ADS14	5.67 (0.33) a	4.33 (1.20) a	8.33 (0.33) a	8.33 (0.33) ab	13.00 (0.58) a	12.00 (0.58) a
IBS8	5.00 (0.00) a	5.67 (0.33) a	8.67 (0.67) a	8.33 (0.33) ab	13.00 (0.58) a	12.00 (0.58) a
OSR7	5.00 (0.00) a	5.33 (0.33) a	8.00 (0.00) a	8.33 (0.33) ab	11.67 (0.33) ab	11.67 (0.33) a
EPR3	5.33 (0.33) a	5.00 (0.00) a	8.00 (0.00) a	8.33 (0.33) ab	11.67 (0.33) ab	12.33 (0.33) a
AT-SKR	5.33 (0.33) a	5.33 (0.33) a	8.33 (0.33) a	9.33 (0.33) a	12.67 (0.33) ab	11.67 (0.33) a
IPR1	5.33 (0.33) a	5.33 (0.33) a	8.33 (0.33) a	8.00 (0.00) ab	11.00 (0.00) ab	12.00 (0.58) a
ILS13	5.33 (0.33) a	5.00 (0.00) a	8.00 (0.00) a	8.00 (0.00) ab	10.67 (0.33) ab	11.33 (0.33) a
AT-IKS	5.00 (0.00) a	5.00 (0.00) a	9.00 (0.58) a	9.00 (0.00) a	13.00 (0.58) a	10.00 (0.00) a
AKR5	5.67 (0.33) a	5.00 (0.00) a	9.00 (0.58) a	8.33 (0.33) ab	10.67 (0.33) ab	11.67 (0.88) a
AT-ILR	5.00 (0.00) a	5.00 (0.00) a	8.00 (0.00) a	8.00 (0.00) ab	11.67 (0.67) ab	12.00 (0.58) a
IGGR11	5.00 (0.00) a	5.67 (0.33) a	8.33 (0.33) a	8.33 (0.33) ab	11.33 (0.33) ab	11.33 (0.33) a
UNS9	5.00 (0.00) a	5.33 (0.33) a	7.67 (0.33) a	8.33 (0.33) ab	11.33 (0.33) ab	11.67 (0.67) a
Mean	5.07	4.88	8.16	7.93	11.09	10.94
MSD ($\alpha = 0.05$)	1.46	2.75	1.81	3.39	4.93	5.40

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. WAP = Weeks After Planting, ST = Sterilized Soil, UNST= Unsterilized Soil.

Table 4.26: Effect of bacteria inoculation on leaf area, leaf colour and leaf spot of maize in both sterilized and unsterilized soil

Weeks	**Leaf area (cm^2)**		**Leaf chlorosis**		**Leaf spot**	
Treatment	ST	UNST	6WAP-ST	6WAP-UNST	ST	UNST
Mz (-ve)	286.57 (7.31) a	**207.90 (38.71) a**	0.33 (0.33) a	0.00 (0.00) a	1.00 (0.58) a	0.33 (0.33) ab
Mz + NPK (+ve)	**316.73 (14.08) a**	291.33 (70.99) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	**5.00 (0.00) a**
EPR2	278 (13.45) a	291 (11.24) a	0.00 (0.00) a	0.33 (0.33) a	0.00 (0.00) b	1.00 (0.00) bc
EBS8	227.73 (25.27) a	257.73 (3.72) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.00 (0.00) bc
EPR4	302.83 (13.80) a	299.03 (36.82) a	0.00 (0.00) a	0.33 (0.33) a	0.00 (0.00) b	0.33 (0.33) c
ABS6	260.13 (23.64) a	265.50 (14.76) a	0.33 (0.33) a	0.00 (0.00) a	0.00 (0.00) b	0.67 (0.33) c
EPR7	292.90 (9.82) a	267.17 (19.24) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.67 (0.33) c
TDS9	232.47 (21.72) a	255.17 (10.25) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.00 (0.00) bc
IGBR11	282.57 (7.31) a	**382.90 (56.59) a**	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	0.67 (0.67) c
ADS14	213.90 (107.81) a	318.90 (48.02) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	0.33 (0.33) c
IBS8	283.93 (22.33) a	362.03 (34.01) a	0.67 (0.67) a	0.33 (0.33) a	0.00 (0.00) b	1.00 (1.00) bc
OSR7	294.87 (25.45) a	300.63 (35.65) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.00 (0.58) bc
EPR3	265.87 (9.19) a	336.17 (3.19) a	0.67 (0.67) a	0.00 (0.00) a	0.00 (0.00) b	1.67 (0.33) bc
AT-SKR	235.50 (35.42) a	289.07 (25.21) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.67 (0.67) bc
IPR1	253.03 (11.26) a	281.57 (21.33) a	033 (0.33) a	0.00 (0.00) a	0.00 (0.00) b	1.33 (0.33) bc
ILS13	**308.03 (16.43) a**	237.83 (31.54) a	0.33 (0.33) a	0.33 (0.33) a	0.00 (0.00) b	1.33 (0.88) bc
AT-IKS	289.09 (54.45) a	255.83 (11.18) a	0.33 (0.33) a	0.00 (0.00) a	0.00 (0.00) b	2.00 (0.58) bc
AKR5	271.47 (31.24) a	275.93 (52.67) a	0.67 (0.33) a	0.00 (0.00) a	0.00 (0.00) b	0.67 (0.67) c
AT-ILR	287.10 (8.87) a	354.37 (42.90) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	1.00 (0.58) bc
IGGR11	264.50 (17.93) a	317.80 (16.29) a	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) b	0.67 (0.33) c
UNS9	296.23 (25.79) a	344.27 (22.83) a	0.67 (0.67) a	0.67 (0.67) a	0.00 (0.00) b	1.00 (0.58) bc
Mean	263.99	283.10	0.17	0.09	0.04	1.29
MSD ($\alpha = 0.05$)	174.33	188.01	1.56	1.07	0.65	2.64

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. WAP = Weeks After Planting, ST = Sterilized Soil, UNST= Unsterilized Soil.

Table 4.27: Effect of bacteria application on dry matter, pH and microbial load of maize in both sterilized and unsterilized soil

Weeks	**Dry matter**		**pH**		**Bacteria load (10^{-6} CFU/g soil)**	
Treatment	ST	UNST	ST	UNST	ST	UNST
Mz (-ve)	1.72 (0.38) h	3.97 (0.19) d-g	6.43 (0.03) d-f	6.21 (0.12) h-k	**43.00 (4.73) h**	390.67 (51.41) e-i
Mz + NPK (+ve)	5.27 (0.38) b	5.07 (1.45) c-e	6.29 (0.01) gh	5.84 (0.09) l	58.67 (14.71) gh	**56.67 (2.96) kl**
EPR2	5.82 (0.15) b	3.64 (0.02) d-g	6.66 (0.03) ab	6.63 (0.03) b-f	216.00 (4.62) b-d	589.00 (23.63) a-d
EBS8	5.50 (0.15) b	**7.77 (0.48) ab**	6.59 (0.05) bc	6.23 (0.07) h-k	152.67 (12.98) b-g	147.00 (32.69) j-l
EPR4	4.57 (0.09) c	3.74 (0.06) d-g	6.59 (0.05) bc	6.47 (0.03) d-i	93.67 (8.88) e-h	551.00 (56.15) a-e
ABS6	2.47 (0.09) de	2.23 (0.12) gh	6.62 (0.02) ab	6.47 (0.03) d-i	139.67 (10.42) c-g	319.00 (42.53) g-k
EPR7	4.53 (0.03) c	4.23 (0.03) c-g	6.60 (0.00) bc	6.71 (0.06) b-e	**227.33 (30.64) bc**	627.00 (3.00) ab
TDS9	3.34 (0.18) fg	3.39 (0.15) e-h	6.53 (0.03) b-d	6.88 (0.06) ab	217.67 (27.35) b-d	**692.33 (18.98) a**
IGBR11	3.57 (0.03) cd	2.46 (0.07) f-h	6.30 (0.00) gh	6.57 (0.03) c-f	118.33 (9.39) e-h	536.67 (19.88) a-f
ADS14	5.37 (0.12) b	2.34 (0.14) f-h	6.33 (0.03) fg	6.20 (0.06) i-k	162.00 (3.06) b-f	318.33 (7.84) g-k
IBS8	5.21 (0.12) b	**6.28 (0.12) bc**	6.40 (0.00) e-g	6.17 (0.03) jk	130.67 (5.81) d-h	445.67 (64.71) b-h
OSR7	**6.42 (0.26) a**	3.54 (0.14) d-g	**6.73 (0.03) a**	6.37 (0.03) f-j	139.67 (10.42) c-g	232.33 (38.02) i-l
EPR3	3.58 (0.24) cd	4.19 (0.10) e-g	6.40 (0.00) e-g	6.07 (0.03) kl	241.33 (22.19) b	512.33 (69.17) a-g
AT-SKR	3.63 (0.23) cd	4.43 (0.22) c-f	6.37 (0.03) e-g	6.73 (0.03) b-d	189.00 (24.54) b-e	**669.00 (22.39) a**
IPR1	4.10 (0.78) c	4.11 (0.10) d-g	6.10 (0.00) ij	6.83 (0.03) a-c	57.67 (13.02) gh	330.33 (29.90) g-k
ILS13	3.33 (0.18) cd	3.36 (0.12) d-h	6.10 (0.00) ij	6.53 (0.07) d-g	84.33 (4.18) f-h	413.67 (27.17) c-i
AT-IKS	5.19 (0.10) c	3.42 (0.69) d-h	6.20 (0.00) hi	**7.09 (0.12) a**	117.67 (19.01) e-h	349.00 (31.21) f-i
AKR5	4.63 (0.13) cd	3.54 (0.18) d-h	6.20 (0.00) hi	6.43 (0.03) e-j	150.67 (9.33) b-g	339.00 (15.95) g-j
AT-ILR	4.34 (0.04) cd	5.50 (0.10) cd	6.10 (0.00) ij	6.27 (0.03) g-k	144.00 (12.22) c-g	295.67 (20.75)h-k
IGGR11	2.61 (0.09) de	5.22 (0.04) c-e	6.00 (0.00) jk	6.57 (0.03) c-f	89.67 (7.13) f-h	398.33 (51.20) d-i
UNS9	3.63 (0.09) cd	2.61 (0.01) f-h	5.97 (0.03) k	6.50 (0.00) d-h	94.67 (3.53) e-h	595.33 (39.87)a-c
Mean	6.73	5.92	6.09	6.18	153.45	406.09
MSD ($\alpha = 0.05$)	1.32	2.09	0.13	0.30	95.74	194.86

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences. CFU = Colony Forming Unit. ST = Sterilized Soil, UNST= Unsterilized Soil.

than that of other treatments including the controls in an unsterilized soil (Table 4.24). There was no significant difference in maize stem girth at 4WAP across the treatments. Though, maize treated with IBS8 had the highest stem girth of 1.77 cm which was far better than other treatments including the controls in sterilized soil. Interestingly, stem girth of maize plant fertilized with NPK (positive control) had the highest (2.00 cm) than other treatments in an unsterilized soil (Table 4.24) and this follows the same trend for sterilized soil. Generally, there were no significant ($P < 0.05$) differences across the treatments and also, apart from 2WAP, maize stem girth was more supported and enhanced in sterilized soil than unsterilized soil.

4.9.3 Effect of beneficial bacteria inoculation on maize plant number of leaves

The performance of EPR7, TDS9 and ADS14 treatments on maize plant number of leaves in an unsterilized soil was relatively poor (Table 4.25). Similarly, at 4WAP, there were no significant ($P < 0.05$) differences on leaf number across the treatments. Treatment EBS8, EPR4, EPR7, TDS9, AT-IKS and AKR5 enhanced leaf number at 4WAP in sterilized soil than other treatments. However, UNS9 had a discouraging leaf number as it recorded the lowest (7.67) in sterilized soil, while EPR7 had the lowest (5.23) in an unsterilized soil. Significant ($P < 0.05$) differeces were not observed on leaf number at 6WAP across the treatments. The maize plant treated with NPK (positive control) and maize alone (negative control) had a relatively similar leaf number compared to other treatments. In sterilized soil, ADS14, IBS8 and AT-IKS had the highest number of leaves. Generally, the sterilized soil supported leaf number enhancement than the unsterilized soil (Table 4.25).

4.9.4 Effect of beneficial bacteria inoculation on maize plant leaf area, leaf chlorosis and leaf spot disease

Data on leaf area showed no significant ($P < 0.05$) differences across the treatments. At harvest, and with respect to sterilized soil, the highest (316.73cm^2) leaf area was obtained from maize plant treated with NPK fertilizer, followed by ILS13 (308.03cm^2), while the lowest (213.90cm^2) was recorded from maize plant treated with ADS14. Still on sterilized soil, EPR2, EPR4, EPR7, IGBR11, IBS8, OSR7, EPR3, ILS13, AT-IKS, AKR5, AT-ILR, IGGR11 and UNS9 had the leaf area values greater than that of mean (263.99cm^2) leaf area across the treatments (Table 4.26). In an unsterilized soil, maize plant without any treatment (negative control) had the lowest

(207.90cm^2) leaf area, while the highest (382.90cm^2) was recorded for maize plant treated with IGBR11. Leaf areas were more enhanced in an unsterilized soil compared to sterilized soil.

Leaf chlorosis was observed on maize leaves, though, more obvious on negative control (Maize alone), ABS6, IBS8, EPR3, IPR1, ILS13, AT-IKS, AKR5 and UNS9 in both sterilized and unsterilized soils. Similarly, leaf chorosis was also more pronounced on maize plant treated with EPR2, EPR4, IBS8, ILS13 and UNS9 in an unsterilized soil alone. Considering the leaf spot disease, the effect of treatments was not significantly ($P < 0.05$) different from each other but were observed to be significantly different from maize alone (negative control). However, in an unsterilized soil, leaf spot disease was significantly ($P < 0.05$) different across treatments as the highest (5.00) was obtained from maize plant treated with NPK fertilizer (positive control), while the lowest was recorded in that of maize alone (negative control), EPR4 and ADS14. Genrally, sterilized soil discouraged the occurrence of leaf spot disease on maize plant, while unsterilized soil enhanced the occurrence of leaf spot disease on maize plant (Table 4.26).

4.9.5 Effect of beneficial bacteria on maize plant, dry matter, pH and bacteria load.

Effect of all the treatments on dry matter yield of maize plant were significantly related (Table 4.27). Application of OSR7 treatment to maize plant had pronounced effect that was far better than other treatments including the controls when sterilized soil was used. Low (1.72g) dry matter yield was obtained from the maize alone (negative control) compared to others. Maize alone (negative control) and TDS9 were relatively low and observed to be significantly ($P < 0.05$) different from each other. Maize plant treated with NPK fertilizer (positive control), EPR2, EBS8, ADS14 and IBS8 were significantly ($P < 0.05$) similar in their dry matter yield but were observed to be significantly ($P < 0.05$) different from other treatments. EBS8 treatment was observed to be the best among the treatments with dry matter yield of 7.77g, followed by IBS8 (6.28g) treatment, while ABS6 treatment recorded the lowest (2.23g) in an unsterilized soil (Table 4.27). Generally, the effects of treatments on dry matter yield were more enhanced in sterilized soil compared to unsterilized soil.

The pH of untreated rhizosoil (negative control) was significantly ($P < 0.05$) different from the pH of rhizosoil treated with NPK fertilizer in both sterilized and unsterilized soil. OSR7 treated rhizosoil had the highest (6.73) pH in sterilized soil, while AT-IKS treated rhizosoil had the

highest (7.09) in an unsterilized soil. IPR1, ILS13, AT-IKS, AKR5 and AT-ILR treated rhizosoil pH were similar but were observed to be significantly ($P < 0.05$) different from other treated rhizosoil pH, while in the unsterilized soil, the pH of rhizosoil of treatment EPR4, ABS6, AT-SKR, ILS13 and UNS9 were significantly ($P < 0.05$) different from the pH obtained from untreated soil (negative control), NPK treated soil (positive control), EBS8, ADS14, IBS8, OSR7, EPR3, AKR5, AT-ILR and UNS9 (Table 4.27). In comparison, the mean pH of sterilized soil was low (6.09), while that of unsterilized soil was high (6.18).

When sterilized soil was used, the total bacteria load was increased in all the treatments, though; this effect was less significant in the controls. The bacteria load ranged from the highest (227.33 x 10^{-6} CFU/g soil) for TDS9 to the lowest (43.00 x 10^{-6} CFU/g soil) for NPK treated soil (positive control). The total bacteria load for treatment EPR4, IGGR11 and UNS9 were observed to be low compared to that of EPR2, EBS8, TDS9, ADS14, EPR3, AKR5 and AT-ILR that were observed to be on the high side. In an unsterilized soil, there were significant ($P < 0.05$) differences in the bacteria load across the treatments. The bacteria load of TDS9 had the highest (692.33 x 10^{-6} CFU/g soil), followed by AT-SKR (669.00 x 10^{-6} CFU/g soil), while the lowest (56.67 x 10^{-6} CFU/g soil) was recorded for NPK treated soil (positive control). However, the bacteria load for treatment TDS9 and AT-SKR were not significantly ($P < 0.05$) different from each other as well as ABS6, ADS14 and IPR1 but were observed to be similar to treatment EPR2, EPR4, EPR7, IGGR11, EPR3 and UNS9, while bacteria load for treatment EBS8, ILS13, OSR7, AT-IKS and IGGR11 were related to each other. In comparison, the mean bacteria load in an unsterilized soil was higher (406.09 x 10^{-6} CFU/g soil), than the mean bacteria load ((153.45 x 10^{-6} CFU/g soil) of the sterilized soil (Table 4.27).

Table 4.28: Pearson correlation coefficients on agronomical data obtained from maize plants treated with 19 bacteria isolates in sterilized soil to compare similarities of plant morphology to nutrients uptake.

	PH	SG	LN	LA	LC	BL	Soil pH	DMY	N uptake	P (%)
SG	0.14 ^{ns}									
LN	0.60***	0.01 ^{ns}								
LA	0.03 ^{ns}	0.44**	-0.19 ^{ns}							
LC	0.13 ^{ns}	0.01 ^{ns}	0.09 ^{ns}	0.21 ^{ns}						
ML	0.02 ^{ns}	0.09 ^{ns}	0.11 ^{ns}	-0.28*	-0.40**					
Soil pH	-0.00 ^{ns}	0.10 ^{ns}	0.16 ^{ns}	-0.20 ^{ns}	-0.32*	0.51***				
DMY	0.26 ^{ns}	0.21 ^{ns}	0.12 ^{ns}	0.08 ^{ns}	0.06 ^{ns}	-0.10 ^{ns}	0.12 ^{ns}			
N (%)	0.05 ^{ns}	0.02 ^{ns}	0.24 ^{ns}	0.05 ^{ns}	-0.40**	0.15 ^{ns}	0.10 ^{ns}	0.23 ^{ns}		
P (%)	0.05 ^{ns}	0.15 ^{ns}	0.18 ^{ns}	-0.20 ^{ns}	-0.14 ^{ns}	0.25 ^{ns}	0.33*	0.48**	0.32*	
K (%)	0.09 ^{ns}	0.10 ^{ns}	0.33*	-0.24 ^{ns}	-0.08 ^{ns}	0.50***	0.40**	0.29*	0.34*	0.59***

PH = Plant height, SG = Stem girth, LN = Leaf number, LA = Leaf area, LC = Leaf colour, BL = Microbial load, Soil pH,

DMY = Dry matter yield, N (%) = Percentage nitrogen in maize plant, P (%) = Percentage phosphorus in maize plant,

K (%) = Percentage potassium in maize plant

*= Significant at $P < 0.05$
**= Significant at $P < 0.01$
***= Significant at $P < 0.0001$,
ns = not significant at $P < 0.05$, $P < 0.01$, $P < 0.0001$

Table 4.29: Pearson correlation coefficients on agronomical data obtained from maize plants treated with 19 bacteria isolates in an unsterilized soil to compare similarities of plant morphology to nutrients uptake.

	PH	**SG**	**LN**	**LA**	**LC**	**BL**	**Soil pH**	**DMY**	**N (%)**	**P (%)**
SG	0.58***									
LN	0.11 ns	-0.02 ns								
LA	0.01ns	0.01ns	0.13 ns							
LC	-0.01ns	0.03 ns	0.06 ns	0.08 ns						
BL	0.0 ns 5	0.22 ns	-0.10 ns	-0.08 ns	-0.23 ns					
Soil pH	0.01 ns	0.01 ns	-0.25 ns	-0.18 ns	-0.06 ns	0.32*				
DMY	0.01 ns	0.10 ns	0.04 ns	0.08 ns	-0.13 ns	-0.25 ns	-0.28*			
N (%)	-0.08ns	-0.04 ns	0.11ns	-0.10 ns	0.07 ns	-0.49**	-0.33*	0.36**		
P (%)	-0.07 ns	0.03 ns	0.02 ns	-0.02 ns	-0.06 ns	-0.40**	-0.20 ns	0.87***	0.51***	
K (%)	0.07 ns	0.09 ns	0.09 ns	-0.12 ns	-0.07 ns	-0.05 ns	-0.16 ns	0.36**	0.22 ns	0.30*

PH = Plant height, SG = Stem girth, LN = Leaf number, LA = Leaf area, LC = Leaf colour, ML = Microbial load, Soil pH,

DMY = Dry matter yield, N (%) = Percentage nitrogen in maize plant, P (%) = Percentage phosphorus in maize plant,

K (%) = Percentage potassium in maize plant

*= Significant at $P < 0.05$
**= Significant at $P < 0.01$
***= Significant at $P < 0.0001$,
ns = not significant at $P < 0.05$, $P < 0.01$, $P < 0.0001$

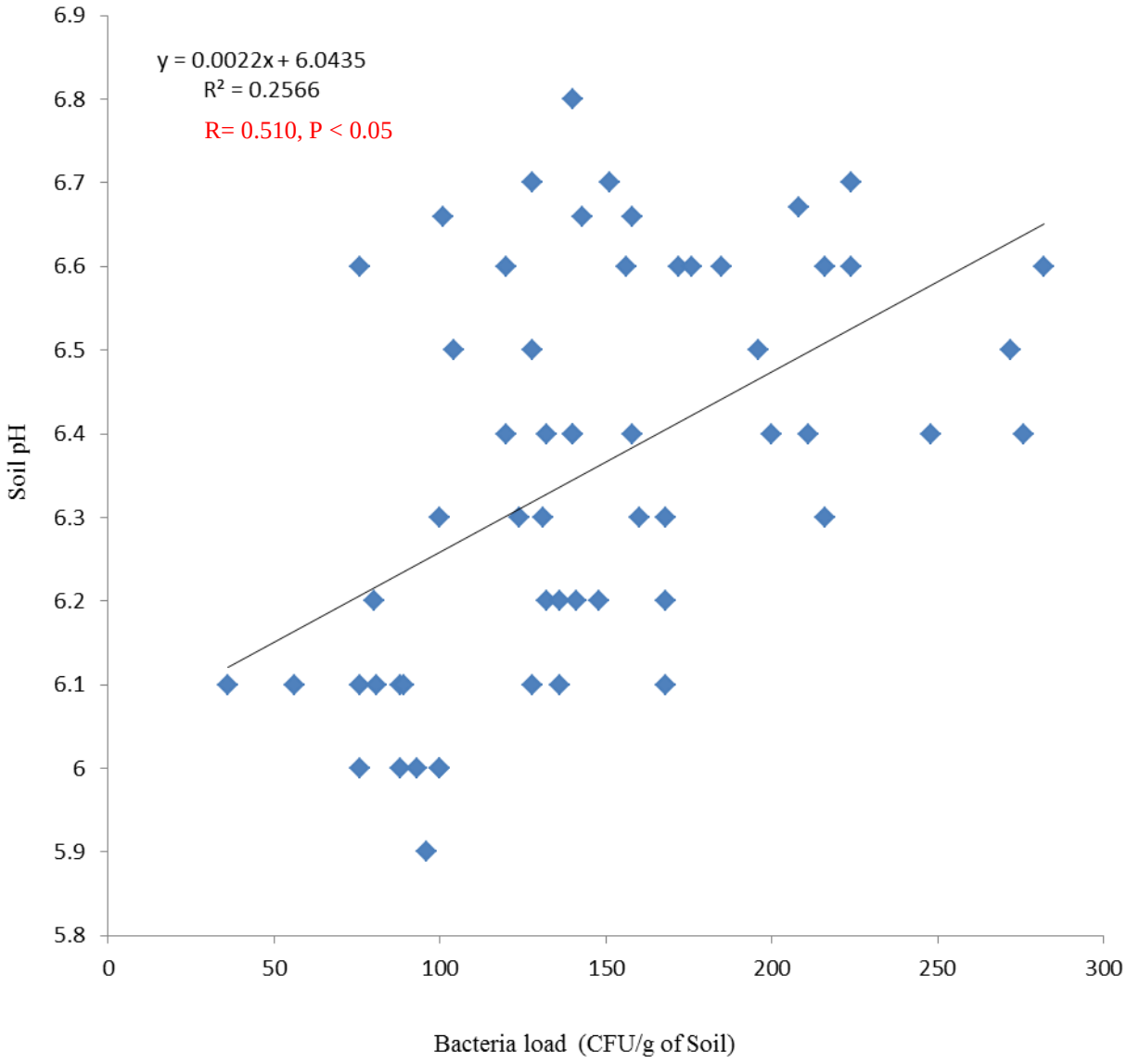


Figure 4.6: Relationship of sterilized soil pH and bacteria load. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the microbial load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

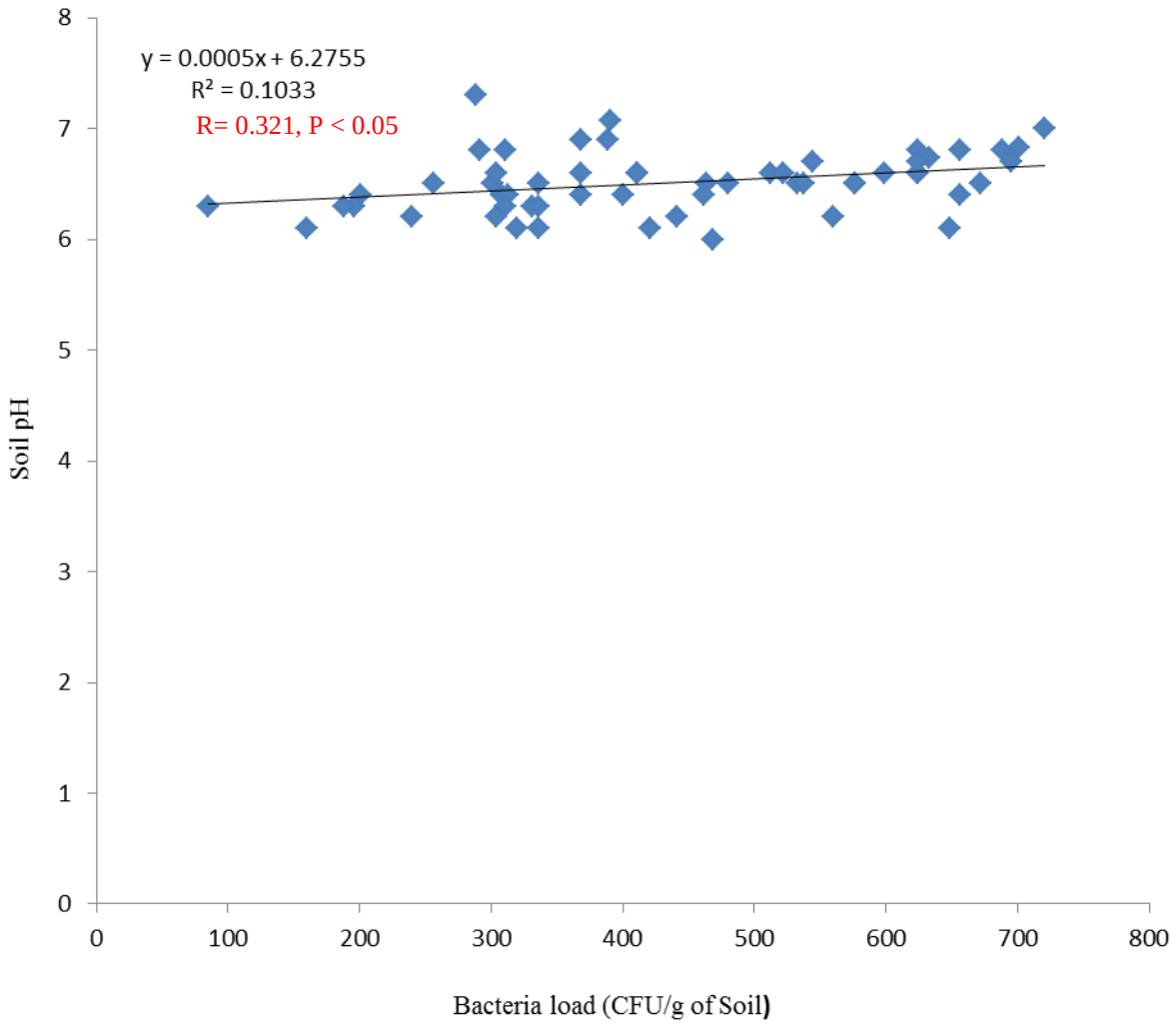


Figure 4.7: Relationship of an unsterilized soil pH and bacteria load. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the microbial load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

4.9.6 Relationship of maize plant morphology and nutrient uptake.

With respect to sterilized soil, there was strong positive correlation ($r = 0.60$, $P < 0.0001$) between plant height and leaf number. Positive correlation ($r = 0.44$, $P < 0.01$) was also found between stem girth and leaf area (Table 4.28). Significant positive correlation were observed between bacterial load, soil pH ($r = 0.51$, $P < 0.05$; Figure 4.6) and uptake of potassium ($r = 0.50$, $P < 0.05$; Table 4.30; Figure 4.11), while nitrogen (Figure 4.9) and phosphorus (Figure 4.10) uptake were not significantly correlated to bacteria load. Phosphorus ($r = 0.33$, $P < 0.05$; Table 4.30) and potassium ($r = 0.40$, $P < 0.05$; Table 4.30) uptake were weakly correlated to soil pH. Likewise, dry matter yield was observed to be positively correlated with phosphorus ($r = 0.48$, $P < 0.01$; Table 4.31) and weakly correlated to potassium ($r = 0.29$, $P <0.05$; Table 4.30). Positive correlation existed between nitrogen, phosphorus and potassium (Table 4.28).

Based on the parameters obtained from maize planted in an unsterilized soil, plant height positively correlated ($r = 0.58$, $P < 0.0001$) with stem girth. Bacteria load was observed to be weakly correlated with soil pH ($r = 0.32$, $P < 0.05$; Table 4.29; Figure 4.7). Also, weak negative correlation was found between bacteria load, nitrogen uptake (($r = -0.49$, $P < 0.05$; Table 4.29; Figure 4.12) and phosphorus ($r = -0.40$, $P < 0.05$; Table 4.30; Figure 4.13) uptake. There was no significant correlation between bacteria load and potassium (Table 4.29; Figure 4.14). Nitrogen, phosphorus and potassium were positively correlated to dry matter yield. Similarly, positive correlation was observed between nitrogen and phosphorus uptake. Soil pH of unsterilized soil for each treatment was negatively correlated to dry matter yield and nitrogen uptake. Other variables were not significant to each other, while some were negatively correlated.

4.9.7 Effect of bacteria isolates on nitrogen uptake in maize

Nitrogen uptake in table 4.30 showed that there were significant differences across the treatments when sterilized soil was used to carry out the experiment. Interestingly, maize plant treated with ADS14 had the highest nutrient compared to other treated maize plant including the positive

Table 4.30: Effect of soil bacteria isolates on maize nutrient uptake

Weeks	**Nitrogen (%)**		**Phosphorus (%)**		**Potassium (%)**	
Treatment	ST	UNST	ST	UNST	ST	UNST
Mz (-ve)	1.32 (0.06) de	1.86 (0.03) b-d	0.29 (0.01) j	0.66 (0.01) c-e	1.74 (0.12) e-g	2.49 (0.72) c-e
Mz + NPK (+ve)	3.98 (0.68) ab	4.05 (0.81) ab	0.75 (0.09) b-e	0.80 (0.01) c	4.08 (0.45) b	2.39 (0.24) c-e
EPR2	2.48 (0.39) b-d	1.35 (0.20) cd	0.72 (0.05) c-e	0.44 (0.04) h-j	7.45 (0.38) a	5.69 (1.24) a
EBS8	2.31 (0.58) b-d	5.52 (0.27) a	0.66 (0.01) c-g	1.56 (0.08) b	3.57 (0.22) b-e	4.80 (0.95) a-c
EPR4	1.44 (0.36) c-e	3.56 (0.32) a-c	0.47 (0.03) g-j	0.45 (0.05) hi	2.39 (0.17) c-f	2.64 (0.36) c-e
ABS6	1.36 (0.01) c-e	2.84 (0.09) a-c	0.45 (0.01) h-j	0.30 (0.01) i-l	1.94 (0.49) ef	1.37 (0.33) de
EPR7	1.54 (0.31) c-e	1.87 (0.71) b-d	0.78 (0.04) b-d	0.62 (0.02) d-g	3.91 (0.45) b-d	1.52 (0.32) de
TDS9	1.94 (0.04) cd	1.02 (0.13) cd	0.41 (0.01) ij	0.50 (0.02) f-h	1.59 (0.07) fg	1.38 (0.29) de
IGBR11	1.32 (0.08) de	2.45 (1.24) b-d	0.56 (0.01) e-i	0.28 (0.02) kl	1.78 (0.17) e-g	1.65 (0.11) de
ADS14	4.53 (0.25) a	2.74 (0.50) bc	0.58 (0.02) e-i	0.26 (0.01) kl	4.72 (0.38) b	2.16 (0.45) c-e
IBS8	2.73 (0.06) b-d	0.96 (0.14) cd	0.67 (0.01) c-f	0.68 (0.02) c-e	2.66 (0.15) c-f	3.40 (0.38) a-d
OSR7	3.02 (0.40) a-c	2.45 (0.74) b-d	0.92 (0.05) b	0.44 (0.03) h-j	4.24 (0.10) bc	3.40 (0.08) a-d
EPR3	2.47 (0.07) b-d	2.63 (0.05) b-d	0.67 (0.01) c-f	0.53 (0.05) e-h	3.53 (0.39) b-e	2.42 (0.26) c-e
AT-SKR	1.45 (0.01) c-e	1.56 (0.69) a-c	0.51 (0.00) f-i	0.48 (0.02) gh	4.22 (0.53) bc	3.90 (0.87) a-d
IPR1	1.92 (0.01) cd	1.95 (0.03) b-d	0.61 (0.01) d-h	0.61 (0.01) d-g	1.40 (0.10) fg	1.91 (0.15) de
ILS13	1.65 (0.35) c-e	1.46 (0.40) b-d	0.42 (0.02) ij	0.29 (0.01) j-l	1.57 (0.18) fg	2.50 (0.30) c-e
AT-IKS	1.36 (0.05) c-e	2.41 (0.23) b-d	0.73 (0.01) b-e	0.48 (0.03) f-h	4.82 (0.39) b	2.88 (0.05) a-d
AKR5	1.60 (0.23) c-e	2.05 (0.30) b-d	0.44 (0.03) h-j	0.40 (0.03) h-k	3.08 (0.71) b-f	2.77 (0.66) c-e
AT-ILR	2.42 (0.28) b-d	3.61 (0.60) a-c	0.85 (0.03) bc	0.69 (0.05) cd	3.88 (0.44) b-d	2.94 (0.44) a-d
IGGR11	2.33 (0.27) b-d	2.01 (0.05) b-d	0.39 (0.03) ij	0.63 (0.00) d-f	2.09 (0.08) d-f	1.82 (0.20) de
UNS9	1.51 (0.38) c-e	1.49 (0.32) b-d	0.47 (0.05) h-j	0.22 (0.02) l	1.38 (0.28) fg	2.86 (0.03) b-d
Mean	2.37	2.41	0.59	0.57	3.23	2.71
MSD ($\alpha = 0.05$)	1.69	2.68	0.19	0.15	1.89	2.82

Values are means (with standard error in parentheses). Different letters within the columns indicate significant differences with respect to the study areas. (Tukey-Kramer HSD test; $\alpha = 0.05$). MSD = Minimum Significant Differences.

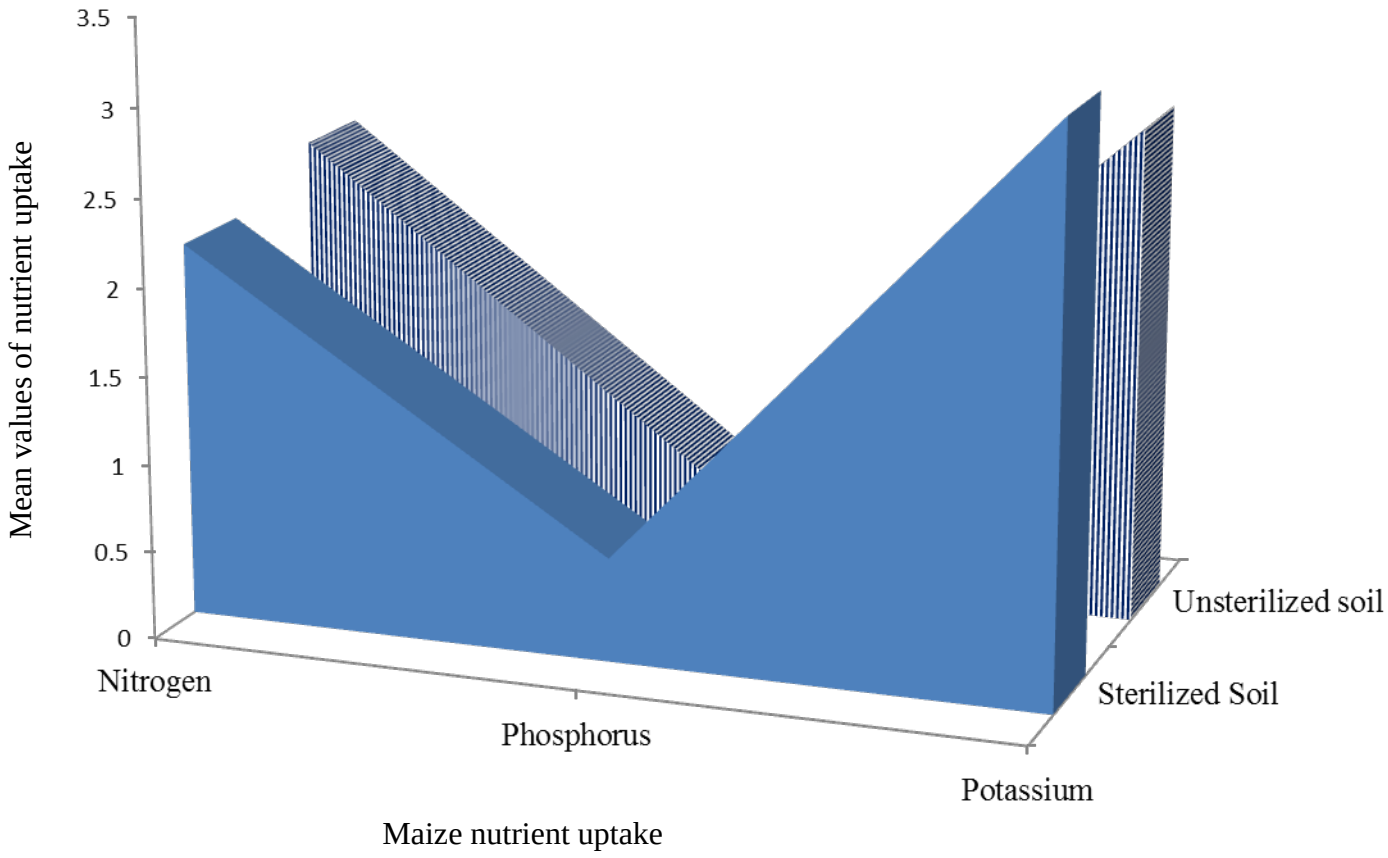


Figure 4.8: Comparison of nutrient uptake in maize plant

control (Maize + NPK). This was followed by maize plant treated with OSR7, while the least was recorded for untreated maize plant (negative control). Values of EPR2, EBS8, ADS14, IBS8, AT-ILR and IGGR11 treatment were observed to be higher than the mean (2.41) value of nitrogen uptake in maize plant grown in sterilized soil. However, there were significant differences in an unsterilized soil. NPK treated (positive control) and untreated (negative control) maize plant were not significantly ($P < 0.05$) different in their nutrient uptake but were observed to be significantly ($P < 0.05$) similar to other treated maize plant. Treatment EBS8 proofed to enhance nitrogen uptake far better even to 3fold than other treatments. EBS8, EPR4, ABS6, IGBR11, ADS14, OSR7, EPR3, AT-IKS and AT-ILR showed significant ($P < 0.05$) effect on nitrogen uptake in maize plant when grown in an unsterilized soil (Table 4.30).

4.9.8 Effect of bacteria isolates on phosphorus uptake in maize

There were significant ($P < 0.05$) differences in phosphorus uptake by the treatments as revealed in table 4.30. When sterilized soil was used, the NPK treated (positive control) and the untreated (negative control) maize plant were observed to be significantly ($P < 0.05$) different from each other in their phosphorus uptake, though, NPK fertilized maize plant (positive control) was observed to be significantly related to EPR2, EBS8, EPR4, IBS8, EPR3, AT-IKS and AT-ILR and also observed to be significantly different from other treatments performances on phosphorus uptake in maize. However, AT-ILR enhanced phosphorus uptake far better than any other treatments. Treatment EPR2, EBS8, EPR7, IBS8, OSR7, EPR3, AT-IKS and AT-ILR had phosphorus value greater than the mean phosphorus value for sterilized soil experiment. When unsterilized soil was used, there was no significant ($P < 0.05$) difference between the NPK treated maize plant (positive control) and untreated maize plant (negative control). ANOVA in table 4.33 revealed that, EBS8, EPR7, IBS8, IPR1, AT-ILR, and IGGR11 enhanced phosphorus uptake better in an unsterilized soil compared to the mean (0.57) phosphorus uptake (Table 4.30).

4.9.9 Effect of bacteria isolates on potassium uptake in maize

EPR2 treatment significantly enhanced potassium uptake in maize plant with high value of 7.45 compared to the controls and other treatments when sterilized soil was used. The potassium uptake in maize treated with NPK fertilizer was significantly different from that of EBS8, EPR7, ADS14, OSR7, EPR3, AT- SKR, AT-IKS, AKR5, AT-ILR and IGGR11 in treated maize

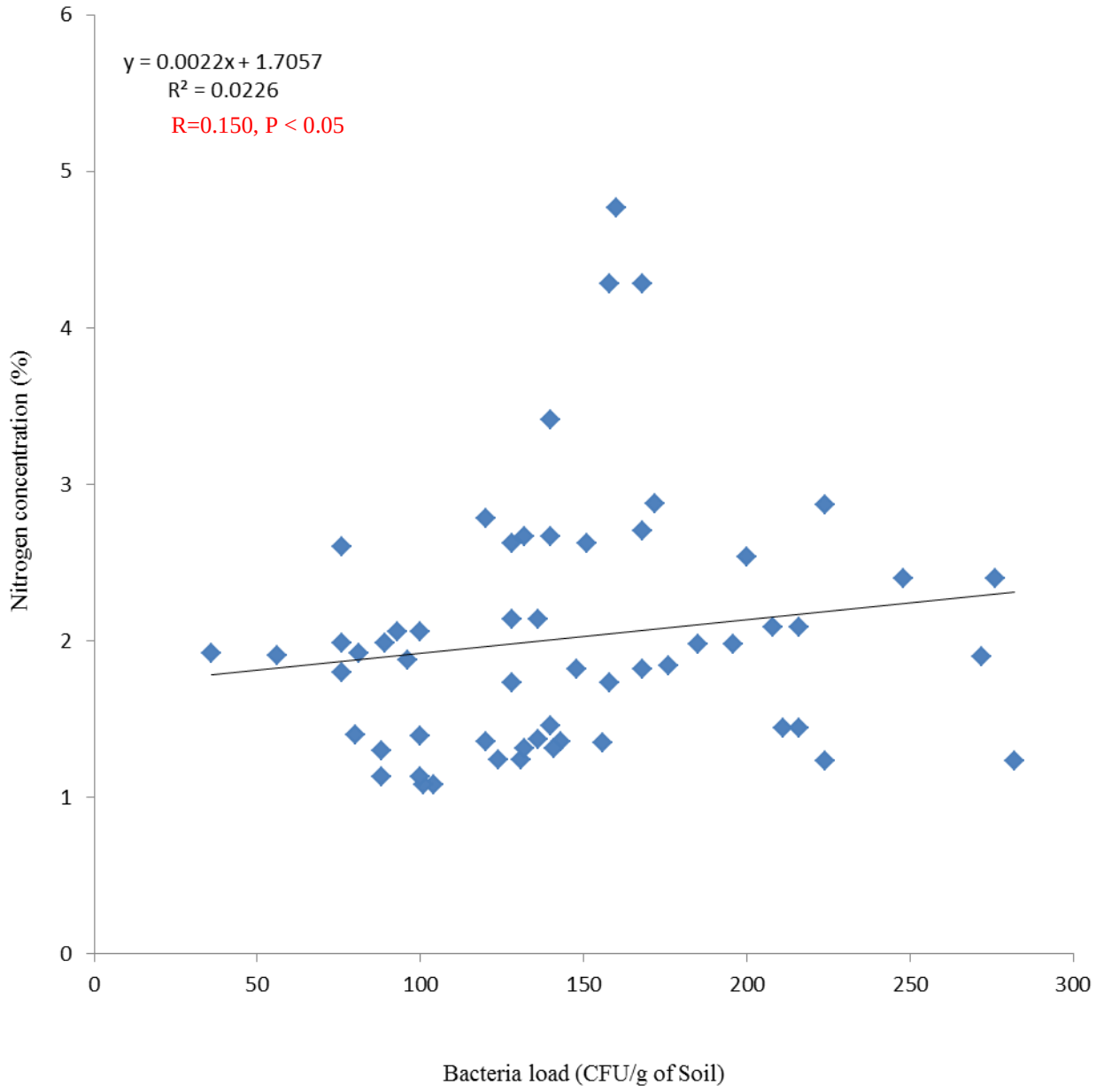


Figure 4.9: Relationship of bacteria load and nitrogen concentration (%) in sterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

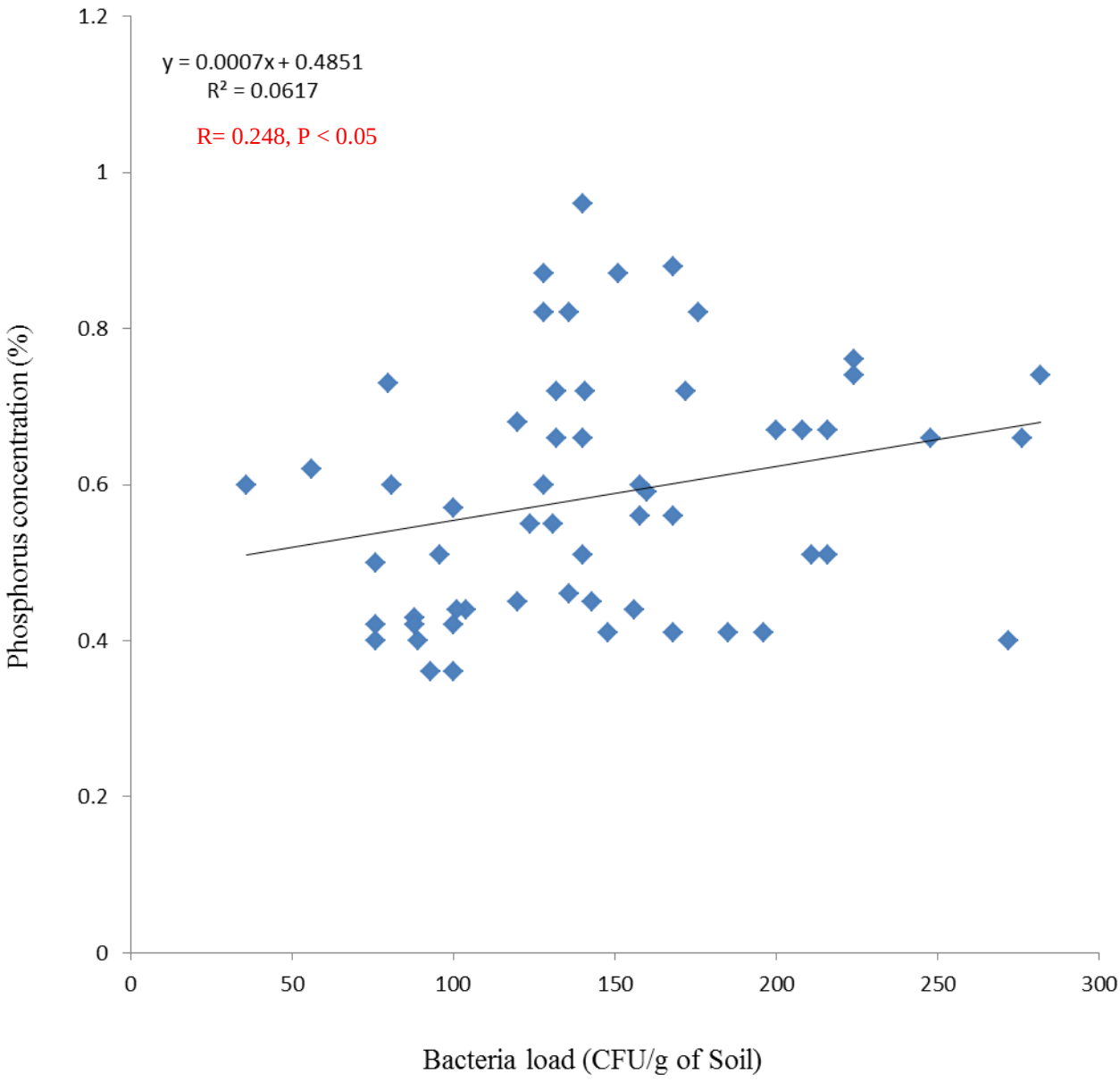


Figure 4.10: Relationship of bacteria load and phosphorus concentration (%) in sterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

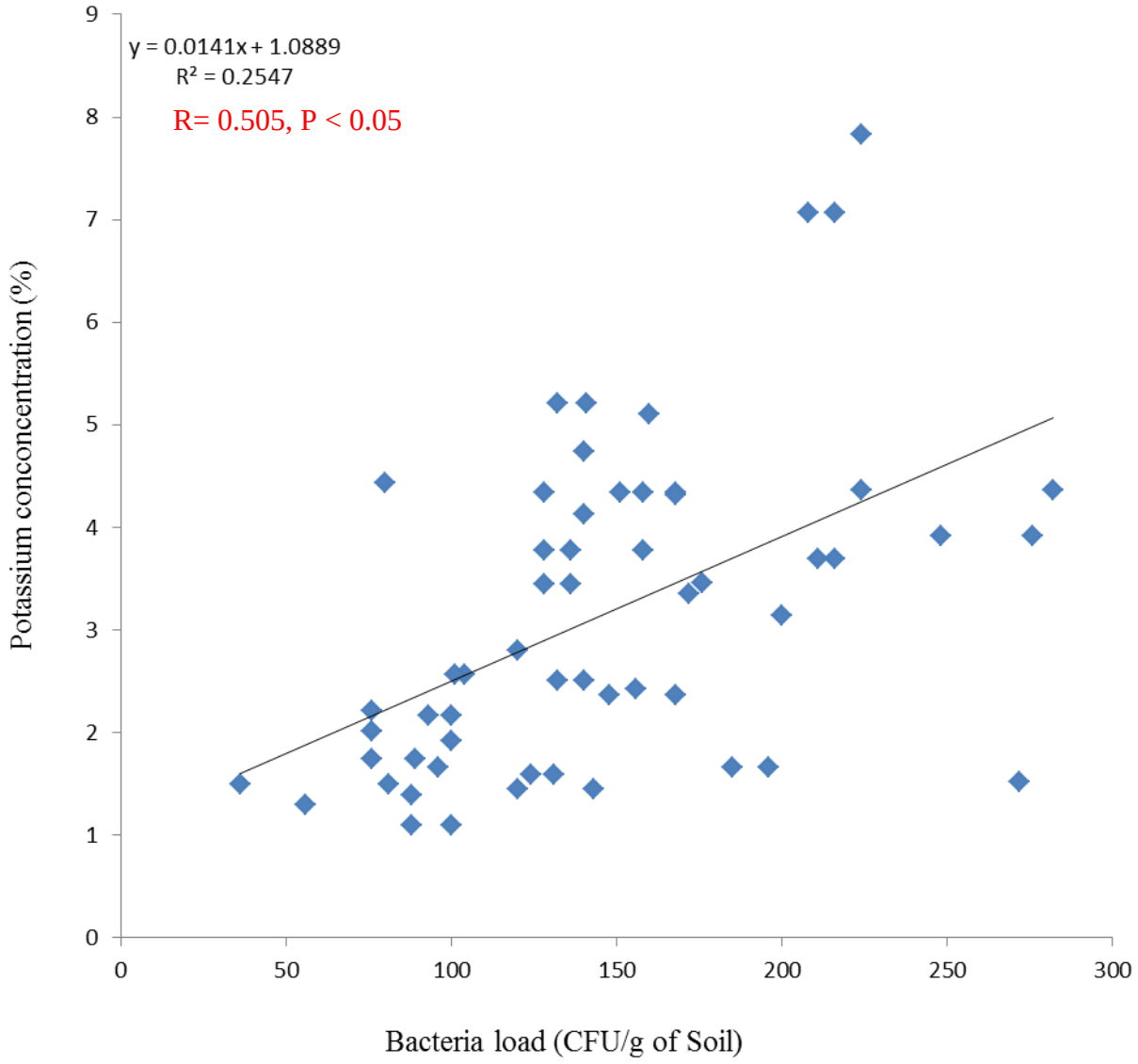


Figure 4.11: Relationship of bacteria load and potassium concentration (%) in sterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

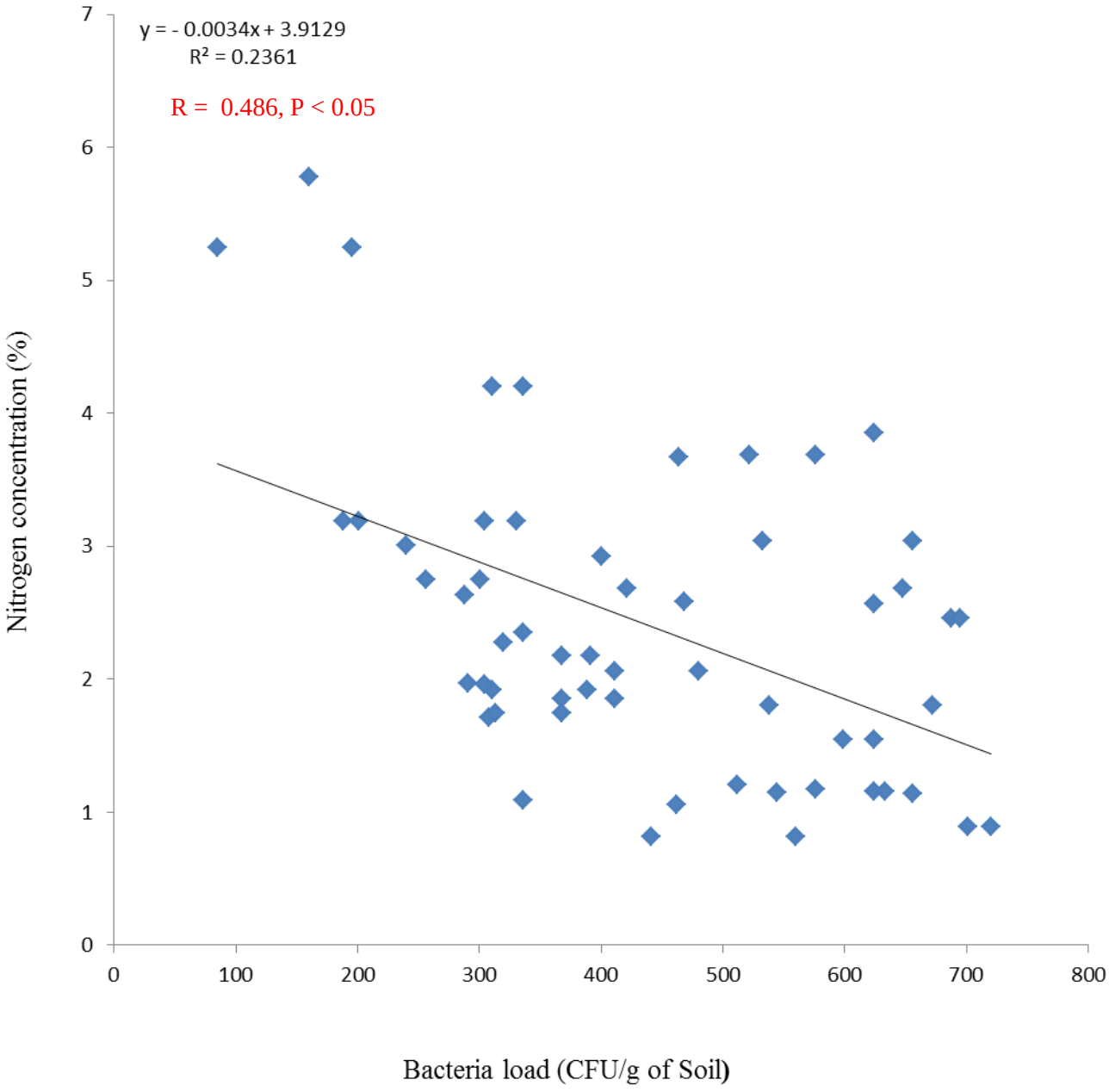


Figure 4.12: Relationship of bacteria load and nitrogen concentration (%) in an unsterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

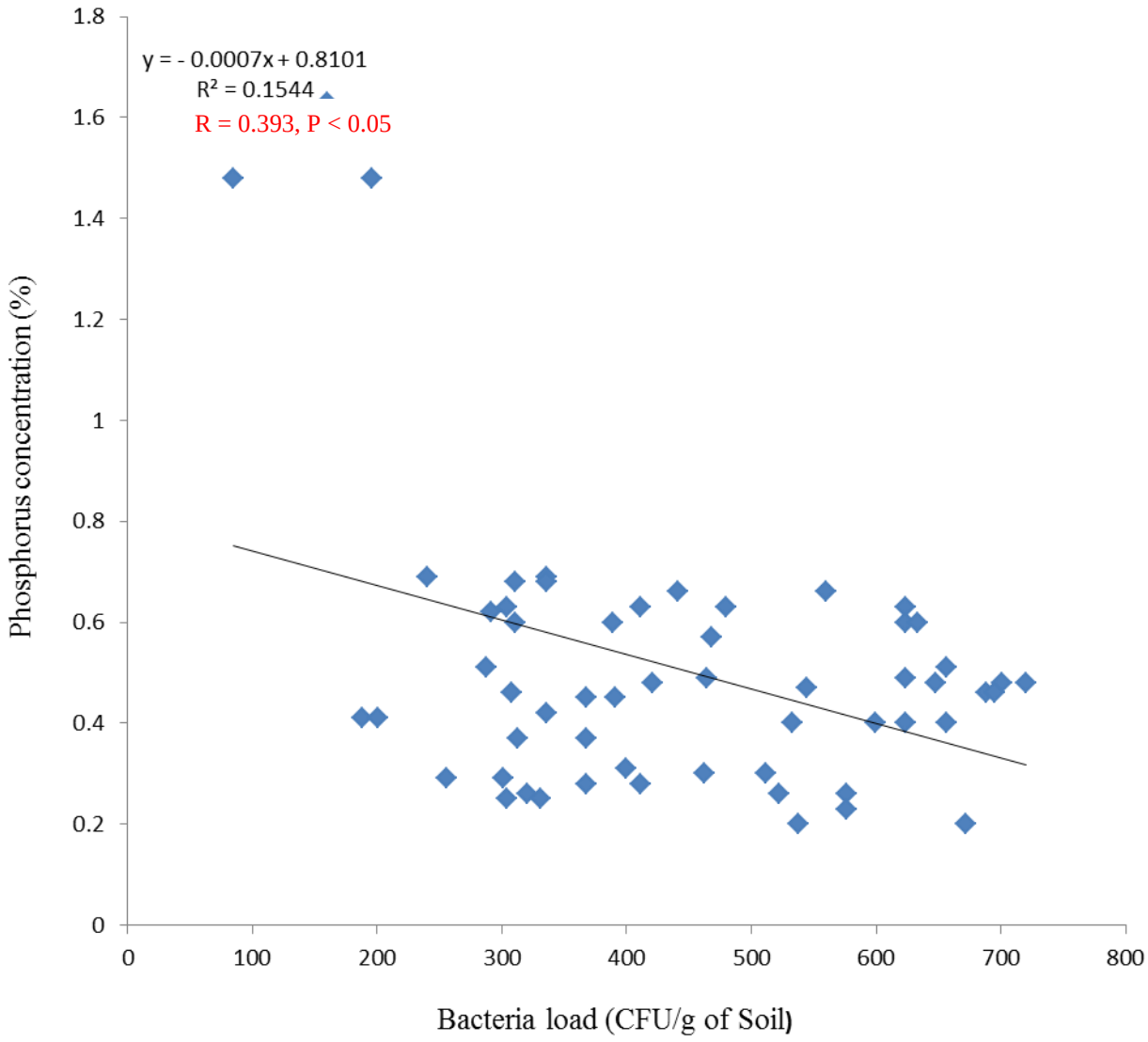


Figure 4.13: Relationship of bacteria load and phosphorus concentration (%) in an unsterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

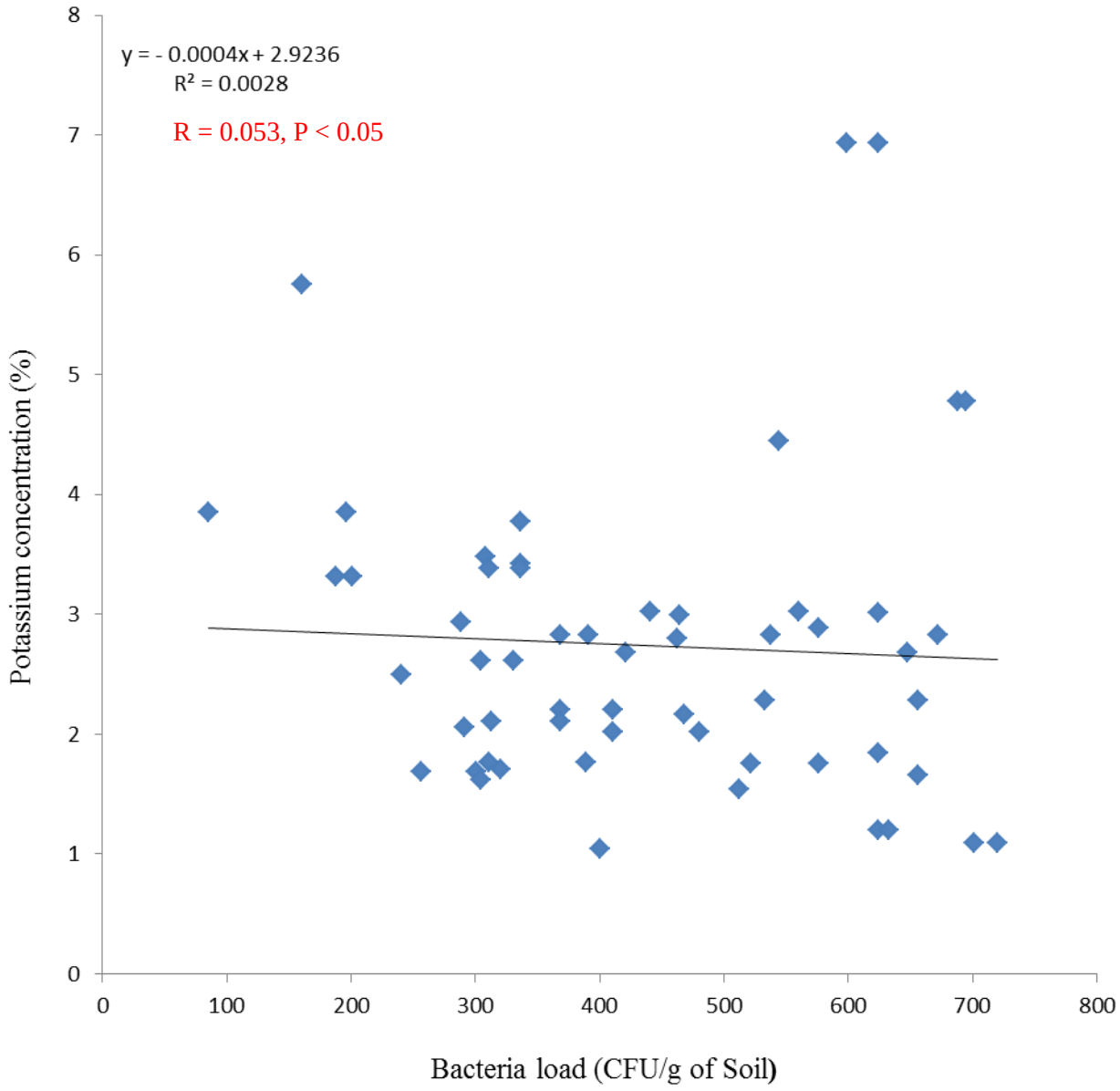


Figure 4.14: Relationship of bacteria load and potassium concentration (%) in an unsterilized soil. Comparisons were made with mean plot values for each variable (n = 19). Linear regression is shown between the soil pH and the bacteria load with statistically significant relationships at $P < 0.05$. CFU = Colony Forming Unit.

plant. However, the potassium uptake in NPK treated maize plant (positive control) was significantly different from that of untreated soil (negative control). Treatment EPR2, EBS8, EPR7, ADS14, OSR7, EPR3, AT-SKR, AT-IKS and AT-ILR were observed with potassium uptake values that were relatively higher than that of the mean (3.23) uptake value for sterilized soil, while EPR2, EBS8, IBS8, OSR7, AT-SKR, AT-IKS, AKR5, AT-ILR and UNS9 treatment recorded potassium uptake higher than the mean (2.71), moreso, sterilized soil supported potassium uptake in maize plant (Table 4.30). In an unsterilized soil, the potassium uptake in NPK treated (positive control) maize plant were significantly similar but were also observed to be significantly ($P < 0.05$) different from EPR 2 treated maize plant. However, the highest (5.69) potassium uptake in maize was recorded for EPR2 when unsterilized soil was used (Table 4.32). Generally, unsterilized soil supported nitrogen uptake in maize plant, while sterilized soil supported potassium uptake in maize plant. Phosphorus uptake was relatively moderate in both sterilized and unsterilized soil (Figure 4.8).

4.9.9.1 Selection of beneficial bacteria isolates

Treatment TDS9, AT-IKS, IPR1, EPR4, IGGR11, OSR7, IBS8, UNS9, ILS13, EPR7, ABS6 and EPR2 were carefully selected based on their performances to enhance maize growth and nutrients (nitrogen, phosphorus and potassium) uptake.

4.9.9.2 DNA extraction and pico-green assay of bacteria isolates

The DNA was isolated from all the twelve (12) cultured isolates. Primer 8F and 1392R typically generated gel electrophoretic DNA band patterns from the PCR product. The banding pattern of DNA was compared with reference strain (Figure 4.15). The isolates showed the same PCR products that were between 1159bp to 1700bp. Picogreen quantification of the DNA generated a curve with $R^2 = 0.999$ (Figure 4.16).

4.9.9.3 DNA Sequences

The sequence results (Appendix 5) are intended to characterize the beneficial bacteria and provide an indication of possible relationships and similarity with reference sequences. The 16S rDNA gene sequences were between 580 to 770bp long with high and low quality DNA

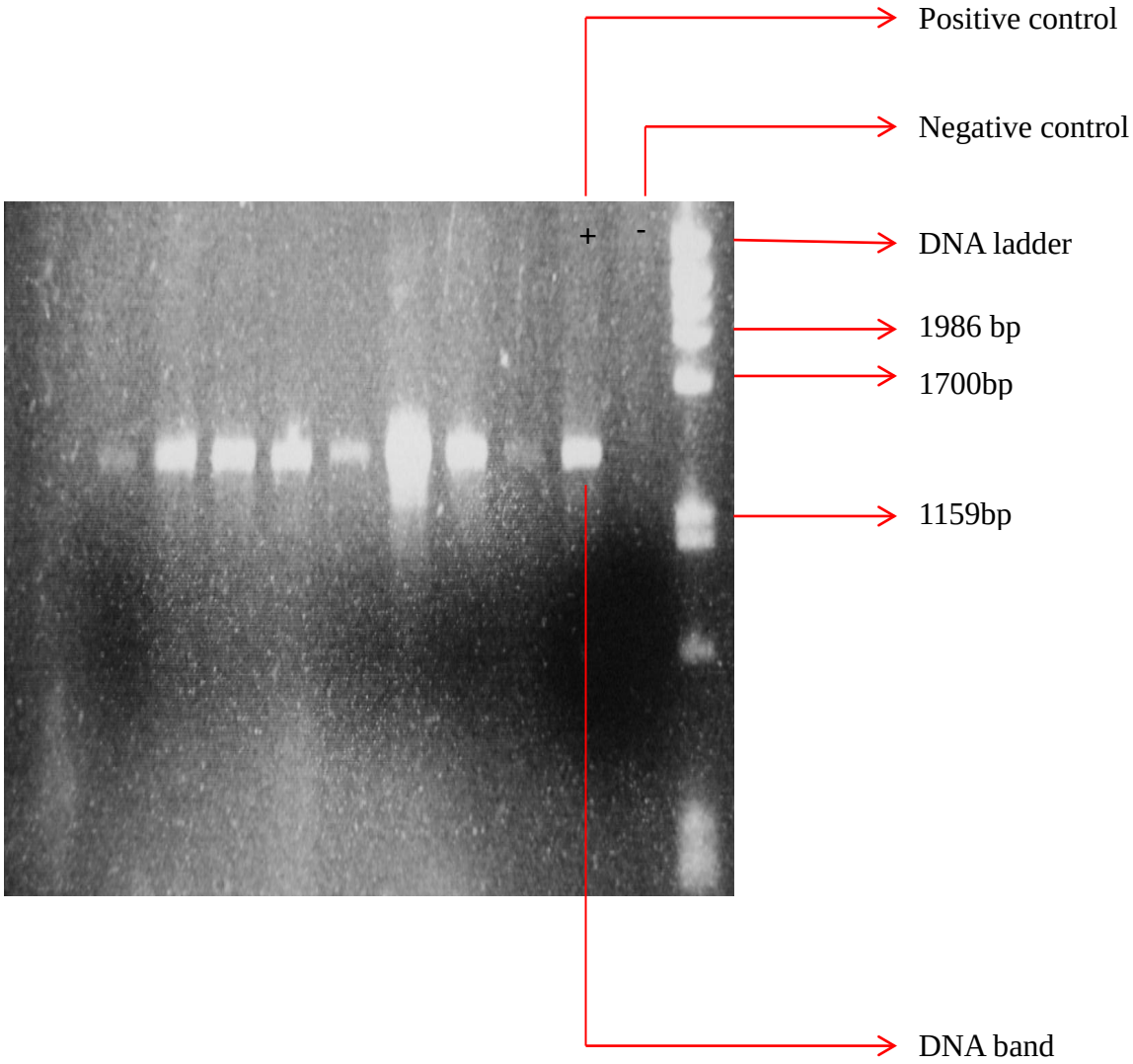
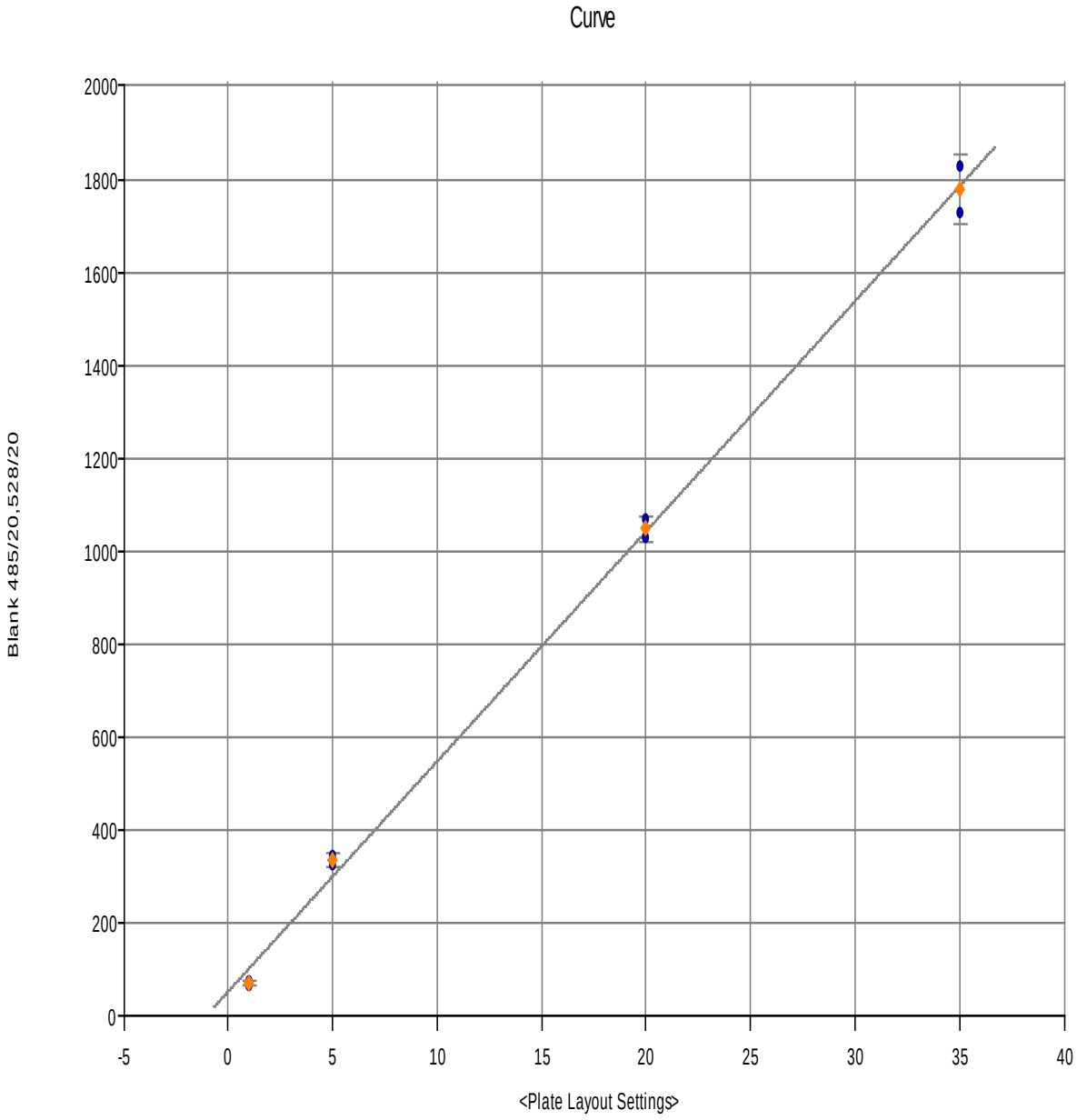


Figure 4.15: 0.5µg / lane, 8 cm length gel, 1X TAE, 7V/cm, 1h. Gel electrophoretic profiles showing presence and absence of phto-beneficial bacteria isolates as visualized by staining of PCR products. bp = base pairs



Curve equation: $Y = A^* X + B$, $R = 0.999$, $R^2 = 0.999$

Figure 4.16: Curve showing PicoGreen DNA assay of bacteria isolates.

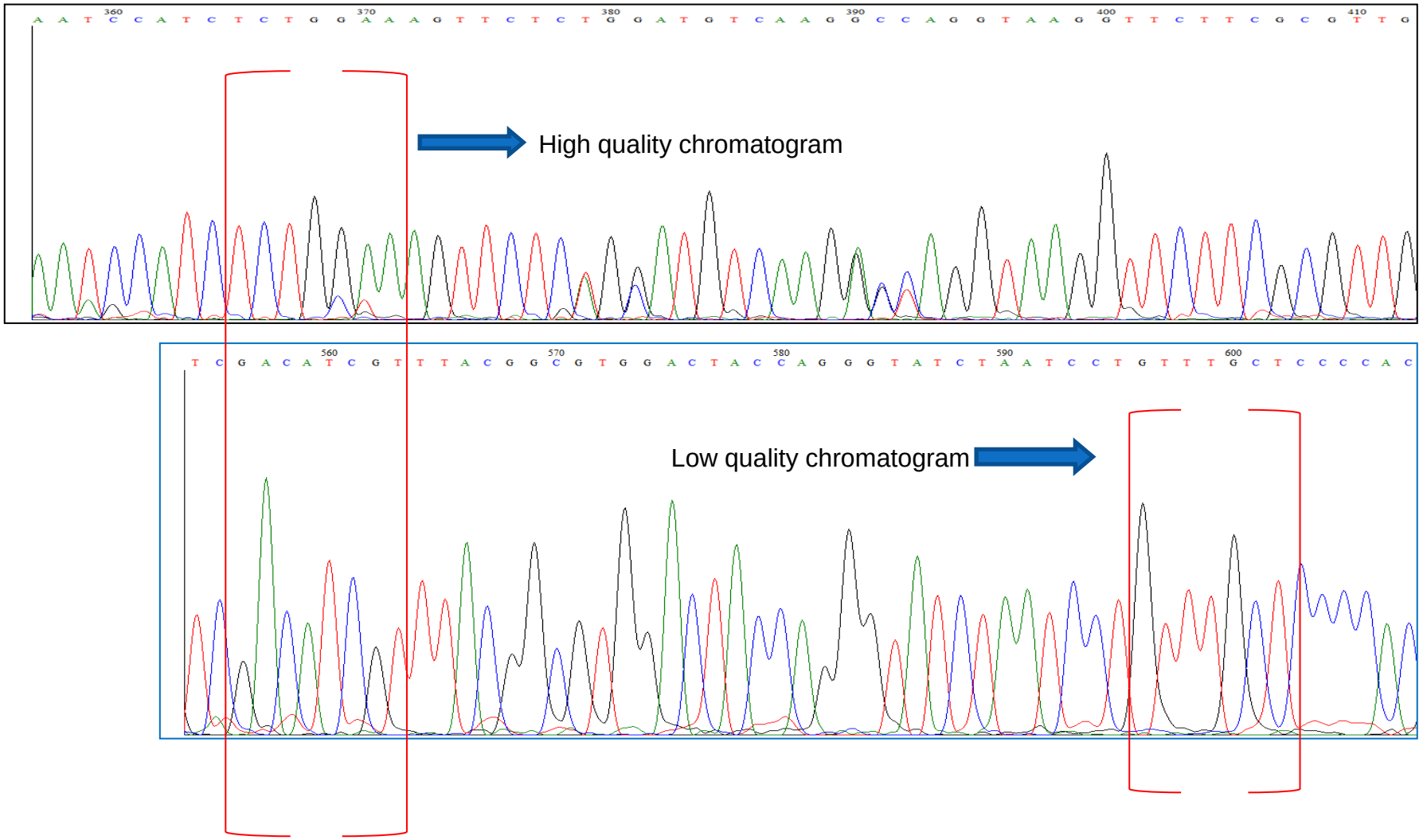


Figure 4.17: High and low quality of DNA sequences chromatogram

Table 4.31: Ribosomal Database Project (RDP) classification

Domain (Bacteria)	
> Phylum (100%) Bacteroidetes	> Phylum (100%) Proteobacteria
>>Class (100%) Flavobacteria	>>Class (100%) Gammaproteobacteria
>>>Order (100%) Flavobacteriales	>>>Order (100%) Enterobacteriales
>>>>Family (100%) Flavobacteriaceae	>>>>Family (100%) Enterobacteriaceae
>>>>>Genus (100%) Myroides	>>>>>Genus (97%) Citrobacter
>>>>>> Myroides sp (EPR4)	**>>>>>> Citrobacter sp (IBS8)**
> Phylum (100%) Bacteroidetes	> Phylum (100%) Firmicutes
>>Class (100%) Flavobacteria	>>Class (100%) Bacilli
>>>Order (100%) Flavobacteriales	>>>Order (100%) Bacillales
>>>>Family (100%) Flavobacteriaceae	>>>>Family (100%) Bacillaceae
>>>>>Genus (100%) Myroides	>>>>>Genus (100%) Bacillus
>>>>>> Myroides sp (AT-IKS)	**>>>>>> Bacillus sp (ILS13)**
> Phylum (100%) Bacteroidetes	> Phylum (100%) Firmicutes
>>Class (100%) Flavobacteria	>>Class (100%) Bacilli
>>>Order (100%) Flavobacteriales	>>>Order (100%) Bacillales
>>>>Family (100%) Flavobacteriaceae	>>>>Family (100%) Bacillaceae
>>>>>Genus (100%) Myroides	>>>>>Genus (100%) Bacillus
>>>>>> **Myroides sp (TDS 9**)	**>>>>>> Bacillus sp (EPR 7)**
> Phylum (100%) Bacteroidetes	> Phylum (100%) Firmicutes
>>Class (100%) Flavobacteria	>>Class (100%) Bacilli
>>>Order (100%) Flavobacteriales	>>>Order (100%) Bacillales
>>>>Family (100%) Flavobacteriaceae	>>>>Family (100%) Bacillaceae
>>>>>Genus (100%) Myroides	>>>>>Genus (100%) Lysinibacillus
>>>>>> **Myroides sp (IPR 1)**	>>>>>> **Lysinibacillus sp (EPR2)**
> Phylum (100%) Proteobacteria	> Phylum (100%) Proteobacteria
>>Class (100%) Gammaproteobacteria	>>Class (100%) Gammaproteobacteria
>>>Order (100%) Enterobacteriales	>>>Order (100%) Xanthomonadales
>>>>Family (100%) Enterobacteriaceae	>>>>Family (100%) Xanthomonaceae
>>>>>Genus (93%) Enterobacter	>>>>>Genus (100%) Stenotrophomonas
>>>>>> **Enterobacter sp (IGGR11)**	>>>>>> **Stenotrophomonas (UNS9)**
> Phylum (100%) Proteobacteria	> Phylum (100%) Proteobacteria
>>Class (100%) Gammaproteobacteria	>>Class (100%) Gammaproteobacteria
>>>Order (100%) Enterobacteriales	>>>Order (100%) Pseudomonadales
>>>>Family (100%) Enterobacteriaceae	>>>>Family (69%) Pseudomonaceae
>>>>>Genus (70%) Enterobacter	>>>>>Genus (26%) Azomonas
>>>>>> **Enterobacter sp (OSR7)**	>>>>>> **Azomoas sp. (ABS 6)**

sequences chromatogram (Figure 4. 17), varied overlaping bases which were moderate but not enough with sufficient phylogenetic information to reliably group strains together and to provide distinguishing and valid measurements of evolutionary relatedness, and thus, phylogenetic placement. Though, the sequencing of PCR amplified 16S rDNA evolutionary similarity was inferred and has revolutionized taxonomy of beneficial bacteria.

4.9.9.4 Phylogenetic analysis

Differences in identification were frequent from Ribosomal Database Project and also compared with that of National Centre for Biotechnology Information (Table 4.31). Identities of closely match genus were obtained. However, blast result proved consistent, reproducible and statistically valid. After comparison of sequences obtained with the registered sequences in the international bank of genes (Gene Bank), the confidence threshold was 80%. The 16SrDNA sequences of the isolates were deposited to Gene bank with accession number in table 4.33. The observed sequences were from three (3) different phyla taxonomic group with seven representative genera (Figure 4.21; Table 4.32).

Phylum proteobacteria dominated (41.67%) with high number of bacteria. The representative genus of phylum Proteobacteria was two strains of *Enterobacter, Citrobacter*, *Stenotrophomonas*, and *Azomonas.* Phyllum Bacteroidetes followed phylum Proteobacteria with 33.33% of representative number of bacteria. The representative genus of Bacteroidetes was four (4) strains of *myroides*. Firmicutes had the lowest (25%) number of bacteria with representative genus of two strains of *Bacillus* and one strain of *Lysinibacillus*. However, based on the genus level, *Myroides* from phylum Bacteroidetes dominated with 33.33% of occurrence, followed by *Enterobacter* and *Bacillus* with 16.67% of occurrence, while the lowest (8.33%) percentage of occurrence was recorded for *Citrobacter, Lysinibacillus, Stenotrophomonas* and *Azomonas* (Figure 4.21).

In an effort to identify isolates to closest similarity match on the phylogenetic tree, molecular phylogeny analysis was conducted. Phylogenetic tree was constructed separately for each of the isolates sequences so as to get a closely related similarity match identity per phyla evolutionary taxonomy group. Good relationship was found between the trees of *Myroides*, though; there were

some differences in branching patterns. The integrity of EPR4 sequences was supported by high (84%) bootstrap value (Figure 4.18 (a)) which in turn formed a phylogenetic similarity with *Myroides odoratus*. Similarly, IPR1 was monophyletic with *Myroides odoratus* (Figure 4.18 (b)) with a bootstrap value of 67%. Likewise, AT-IKS genetic sequences were also observed to be similar to that of *Myroides odoratus* on the phylogenetic tree (Figure 4.18 (c)) with bootstrap value of 64%. In contrast, phylogenetic relationship of TDS9 and *Myroides odoratus* was not supported by a high (59%) bootstrap value (Figure 4.18 (d)).

The 16SrDNA of IGGR11 (*Enteobacter*) determined in this study showed a low (43%) homology with *Enterobacter pyrinus* on phylogenetic tree, while OSR7 (*Enterobacter*) sequences link up phylogenetic relationship with *Enterobacter radicincitans*, though with low bootstrap value of 36%. However, IGGR11 (*Enterobacter*) sequences was not distantly related to OSR7 (*Enterobacter*) sequences on the phylogenetic tree (Figure 4.19 (a)). Observation revealed that genetic sequences of UNS9 (*Stenotrophomonas*) formed a distinct phyletic line closely related to *Stenotrophomonas maltophila* on phylogenetic tree, though supported by low (44%) bootstrap value (Figure 4.19 (b)). IBS8 (*Citrobacter*) sequences was recovered in 55% boostrap analysis and formed a distinct phylogenetic similarity identity with *Citrobacter fameri* (Figure 4.19 (c)). ABS6 (*Azomonas*) was supported by 45% bootstrap value and formed a phylogenetic relationship with *Azomonas macrocytogens* on the phylogenetic tree (Figure 4.19 (d)).

EPR2 (*Lysinibacillus*) appeared to be equally linked to *Lysinibacillus boronitolerans* on the phylognenetic tree supported by 55% bootstrap value (Figure 4.20 (a)). Figure 4.20 (b)) indicated the possibility of ILS13 (*Bacillus*) having the same genetic similarity identity match with *Bacillus niacini* on the phylogenetic tree supported by 71% bootstrap value. However, bootstrap value of ILS13 (*Bacillus*) was significantly different from the bootstrap value of EPR7 sequences on the phylogentic tree. When isolate EPR7 sequences was compared with other *Bacillus* on the phylogenetic tree, it showed sequence identity with *Bacillus aeolius* recovered in 86% of bootstrap value (Figure 4.20 (b)).

The comparison of biochemical characterization and molecular sequencing of isolates does not match to justify the identity of isolates, only with the exception of ILS13 and EPR7, while EPR2 showed family (Bacillaceae) relationship (Table 4.33).

4.9.9.5 Ecological distribution of beneficial bacteria isolates

Data on ecological location of identified beneficial bacteria are presented in table 4.32 and figure 4.22. *Myroides species* were observed to be more dominant as they were found in virtually all the ecological zones in southwestern Nigeria which might therefore justified their potentials to be used alone or in combination with *Stenotrophomonas*, *Enterobacter*, *Citrobacter*, *Lysinibacillus*, *Azomonas* and *Bacillus* as biological fertilizer for maize growth within and across the ecological zones in southwestern Nigeria. *Enterobacter* sp. was ecospecific to derived savannah and lowland rainforest, while *Bacillus* sp. was from derived savannah and freshwater swampy forest. *Citrobacter* sp. had its origin from lowland rainforest, while *Lysinibacillus* sp. and *Azomonas* sp. were from freshwater swampy forest. Observation further showed that, only *Stenotrophomonas* sp. was found in mangrove forest (Table 4.32). Based on the ecological zones (Figure 4.22), fresh water swampy forest had the highest (33.33%) beneficial bacteria isolates, followed by derived savannah (25.00%) and Lowland rainforest (25.00%). The least beneficial bacteria isolate was recorded for guinea savannah (8.33%) and mangrove forest (8.33%).

4.9.9.6 Effect of combined phyto-beneficial beneficial bacteria isolates on maize growth

Based on obtained data so far in this research study, observation showed that NPK chemical fertilizer significantly enhanced maize growth and nutrients uptake, thus, competed with each of the beneficial bacteria isolates on their potentials to enhance maize growth and nutrient uptake in both sterilized and unsterilized soil. However, in order to achieve the aim of this research study which is basically to obtain molecular and beneficial information on the indigenous bacteria of maize plant, with the baseline concept to be used as broad based biofertilizer across and within all the ecological zones in south western Nigeria, thus, substituting NPK chemical fertilizer, the best (Figure 4.23; Table 4.32) and already identified beneficial bacterium from each of the ecological zones, namely; Guinea savannah, Derived savannah, Lowland rainforest, Fresh water swampy forest and Mangrove forest (Figure 4.23) were combined together and evaluated on growth and nutrients (nitrogen, phosphorus and potassium) uptake of maize in an unsterilized soil.

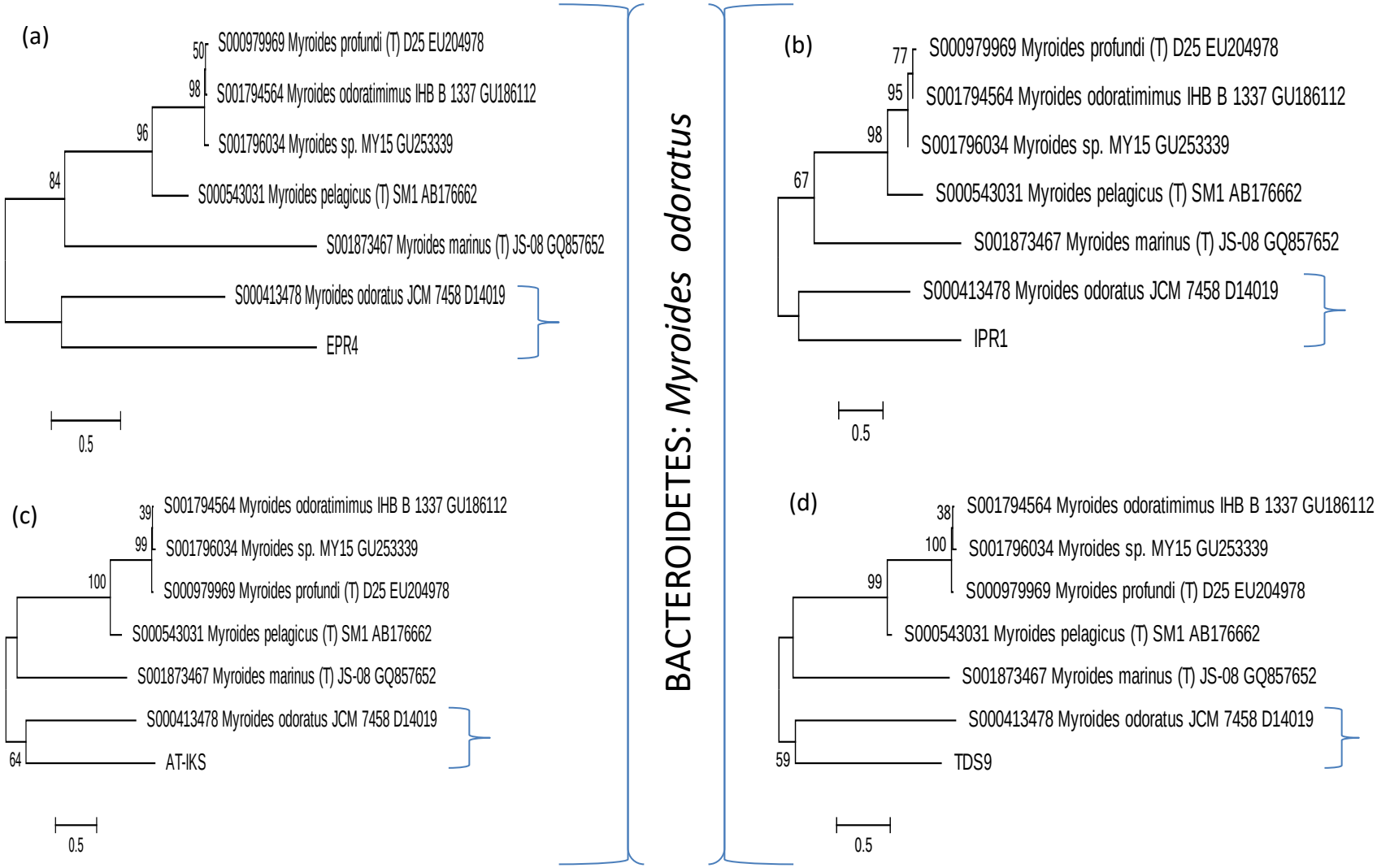


Figure 4.18: Evolutionary history of beneficial bacteria isolates (a) EPR4 (b) IPR1 (c) AT-IKS and (d) TDS 9 based on 16S rDNA gene was inferred using neighbour-joining method. References of the type strains used for comparison are given. Numbers above each node are confidence levels (%) generated from 1,000 bootsrap trees. The scale bar is in fixed nucleotide (0.05) substations per sequence position.

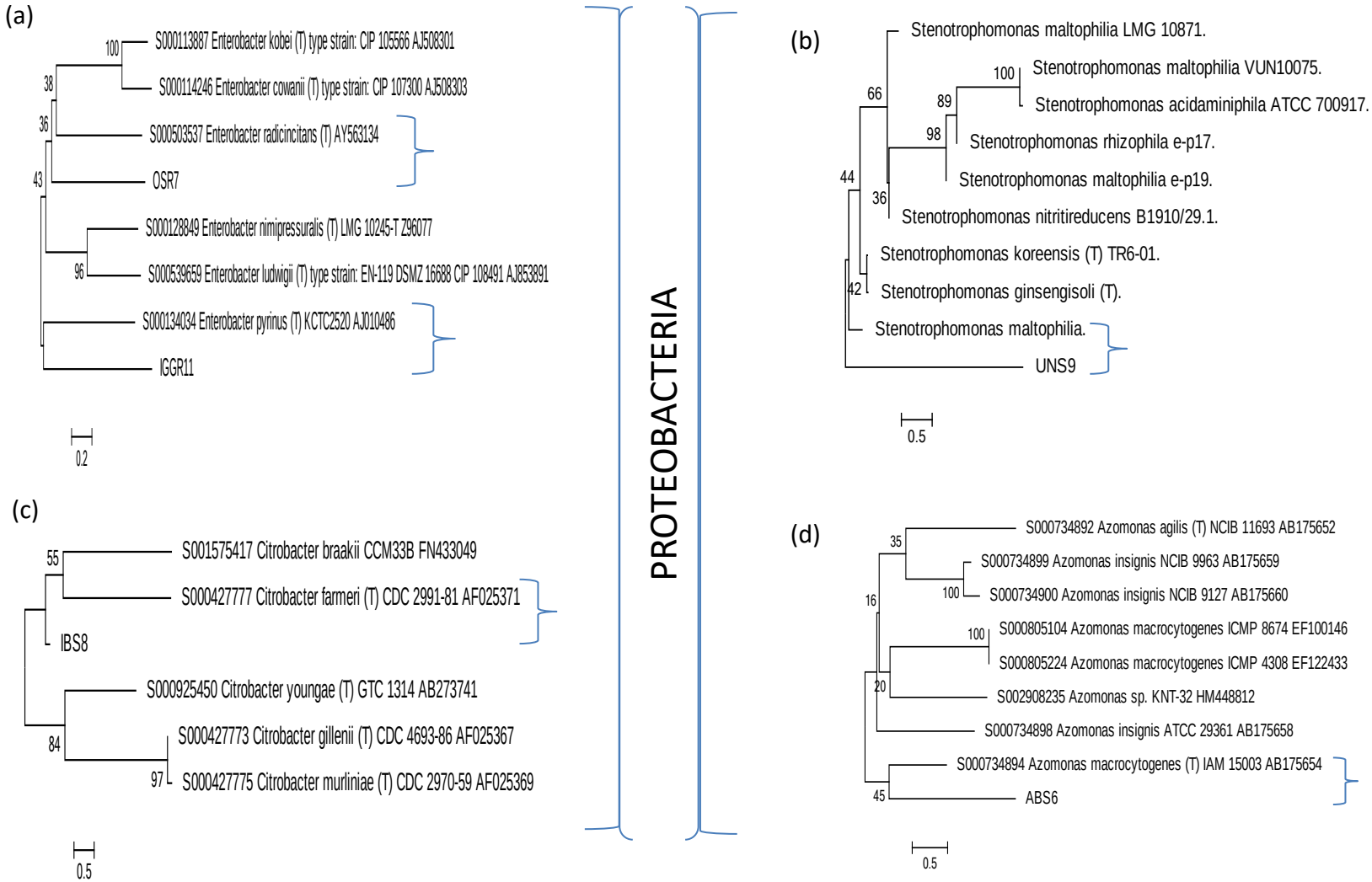


Figure 4.19: Evolutionary history of beneficial bacteria isolates (a) OSR7, IGGR11 (b) UNS9 (c) IBS88 and (d) ABS6 based on 16S rDNA gene was inferred using neighbour-joining method. References of the type strains used for comparison are given. Numbers above each node are confidence levels (%) generated from 1,000 bootsrap trees. The scale bar is in fixed nucleotide (0.05) substations per sequence position.

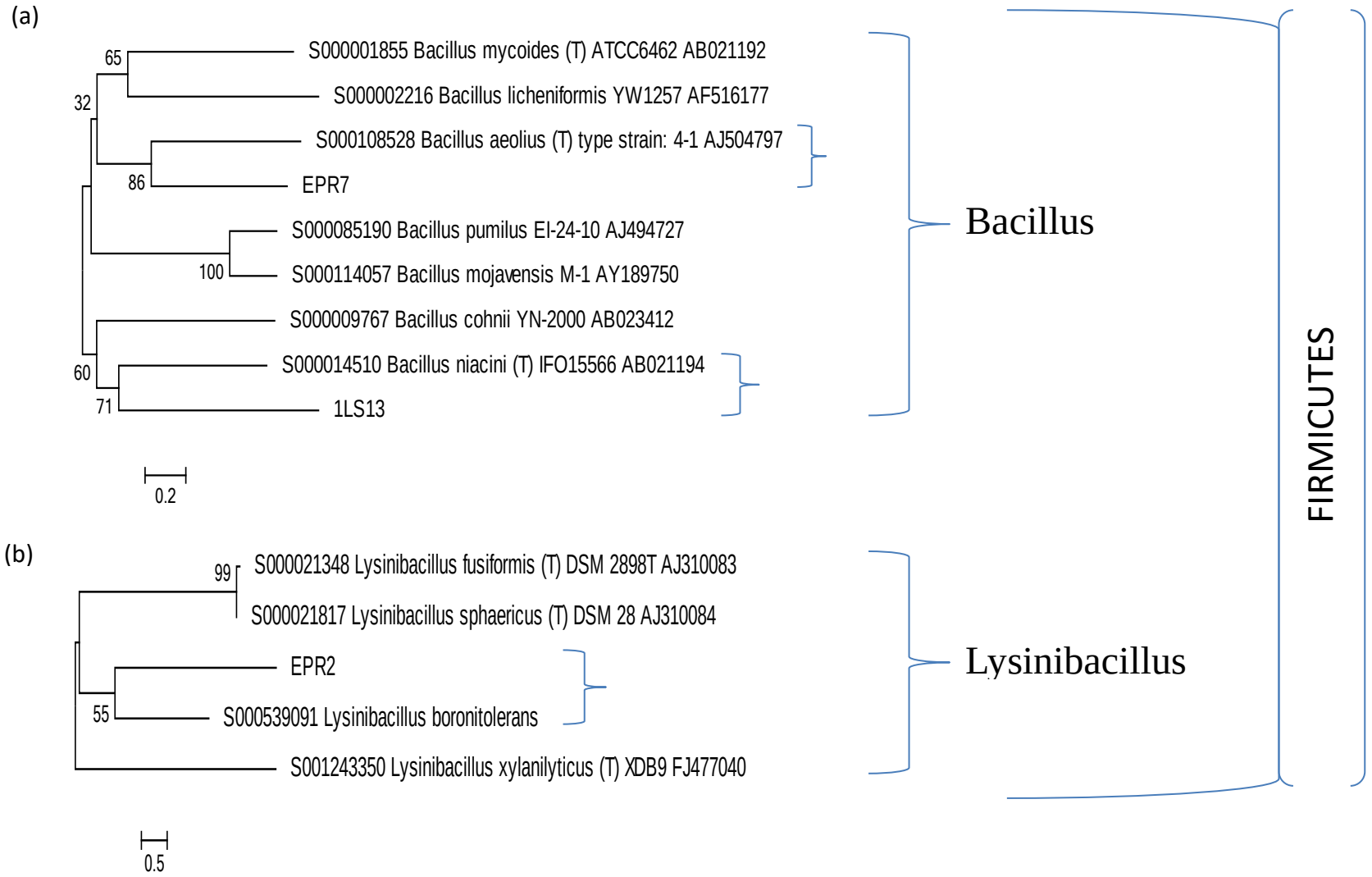


Figure 4.20: Evolutionary history of beneficial bacteria isolates (a) EPR7, ILS13 (b) EPR2 based on 16S rDNA gene was inferred using neighbour-joining method. References of the type strains used for comparison are given. Numbers above each node are confidence levels (%) generated from 1,000 bootsrap trees. The scale bar is in fixed nucleotide (0.05) substations per sequence position.

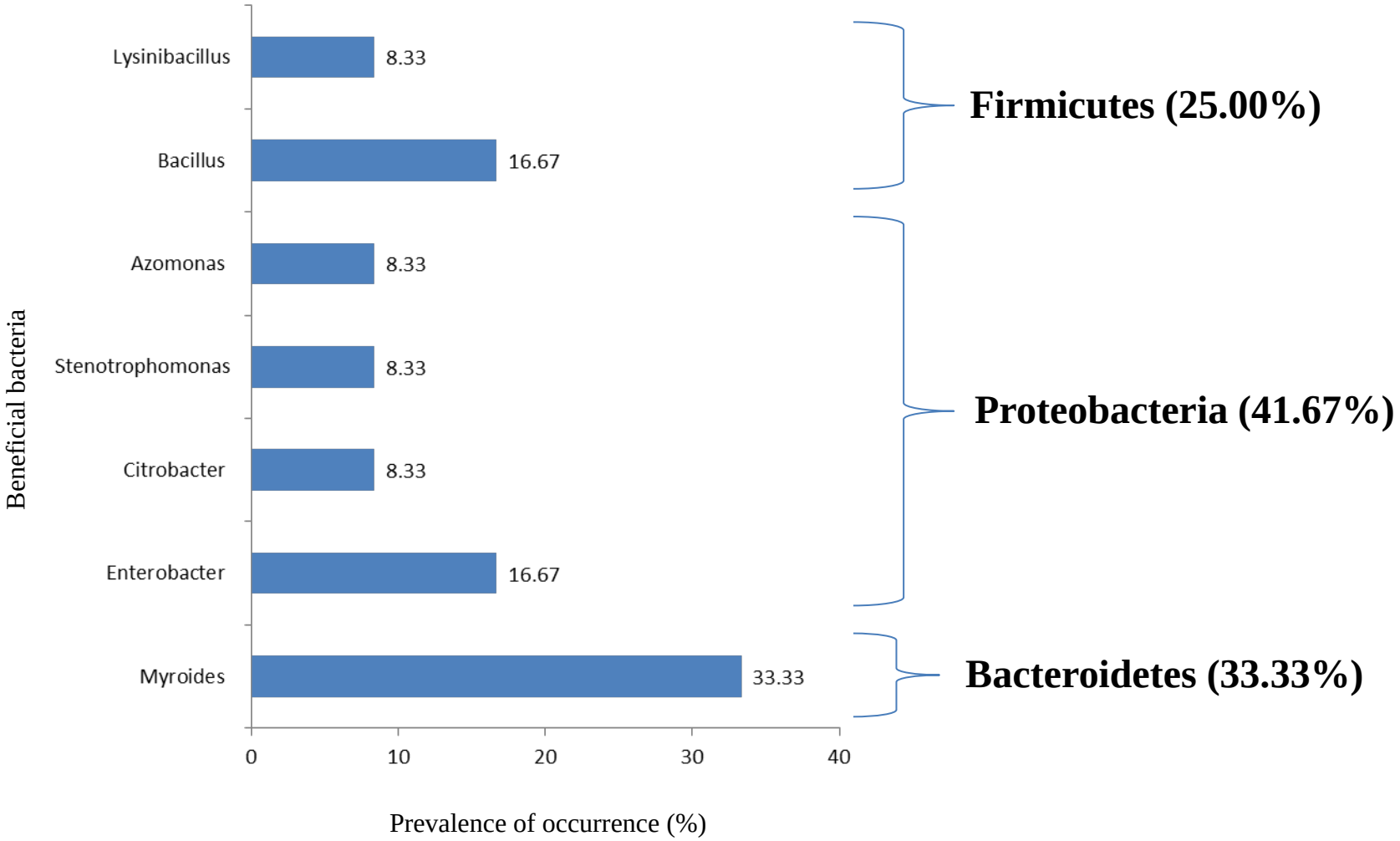


Figure 4.21: Distribution of 16SrDNA identified beneficial bacteria based on the phylla taxonomy

Maize plant treated with combined beneficial bacteria (MZ + CBA) increased maize height compared to other treatments (Figure 4.24), though, not significantly ($P < 0.05$) different from the height of maize plant treated with NPK fertilizer (MZ + NPK) and that of untreated maize plant (MZ alone) at 2 , 4 and 6 Weeks After Planting (WAP). Data in figure 4.25 revealed that there were no significant differences across the treated maize stem girth at 2WAP, 4WAP and 6WAP. However, observation showed that the stem girth of maize plant treated with combined bacteria (MZ + CBA) and NPK (MZ + NPK) fertilizer separately were significantly enhanced far better than that of the stem girth of maize alone at 4WAP and 6WAP. Although, physical observation during the screenhouse study showed that the stem girth of maize plant treated with combined bacteria (MZ + CBA) were more enhanced (Figure 4.25).

Maize plants treated with combined bacteria (MZ + CBA) gave the best leaf number most especially at the time of harvest. There were no significant differences across the treatments at 2WAP and 4WAP but at 6WAP. The leaf number of untreated maize plant (MZ alone) and NPK treated maize plant (MZ + NPK) were observed to be significantly different from the leaf number of maize plant treated with bacteria (MZ + CBA), that is, the combined bacteria significantly increased the leaf number of maize plant compared to other treatments (Figure 4.26). The leaf area was significantly ($P < 0.05$) affected by the treatments. The highest leaf area as at the time of harvest was obtained from maize plant treated with combined bacteria (MZ + CBA) and that of untreated maize plant (MZ alone), though, the leaf area of maize plant treated with combined bacteria (MZ + CBA) and that of untreated maize plant (MZ alone) were not significantly different from each other but were observed to be significantly different from the leaf area of NPK treated maize plant (Figure 4.27).

Leaf chlorosis was consistently observed on untreated maize plant (MZ alone) during the screenhouse study, followed by NPK treated maize plant (MZ + NPK), while little or no leaf chlorosis was observed on maize plant treated with combined bacteria (MZ + CBA). However, ANOVA showed that, the leaf chlorosis on maize plant fertilized with NPK (MZ + NPK) and that of untreated maize plant (Figure 4.28) were not significantly different from each other.

Table 4.32: Identified soil bacteria isolates based on their ecological zone

Bacteria isolates	Guinea Savannah	Derived Savannah	Lowland Rainforest	Fresh Water swampyforest	Mangrove Forest
Genus 1 (*Myroides*)	1 (**TDS9**)	1 (AT-IKS)	1 (IPR1)	1 (EPR4)	
Genus 2 (*Enterobacter*)		1 (IGGR11)	1 (**OSR7**)		
Genus 3 (*Citobacter*)			1 (IBS8)		
Genus 4 (*Stenotrophomonas*)					1 (**UNS9**)
Genus 5 (*Bacillus*)		1 (**ILS13**)		1 (EPR7)	
Genus 6 (*Azomonas*)				1 (ABS6)	
Genus 7 (*Lysinibacillus*)				1 (**EPR2**)	

The **bold** beneficial bacteria isolates were the best in each ecological zone

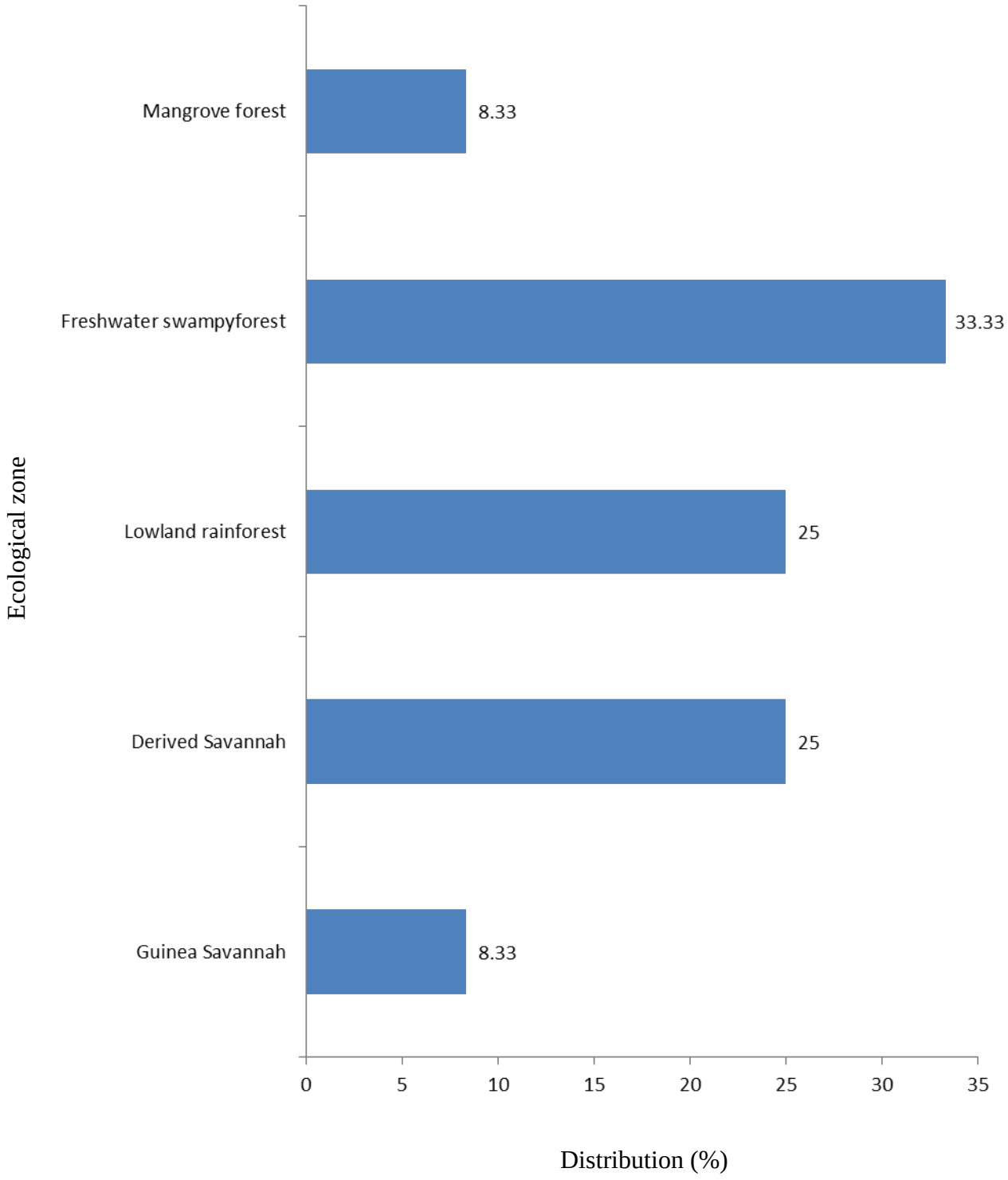
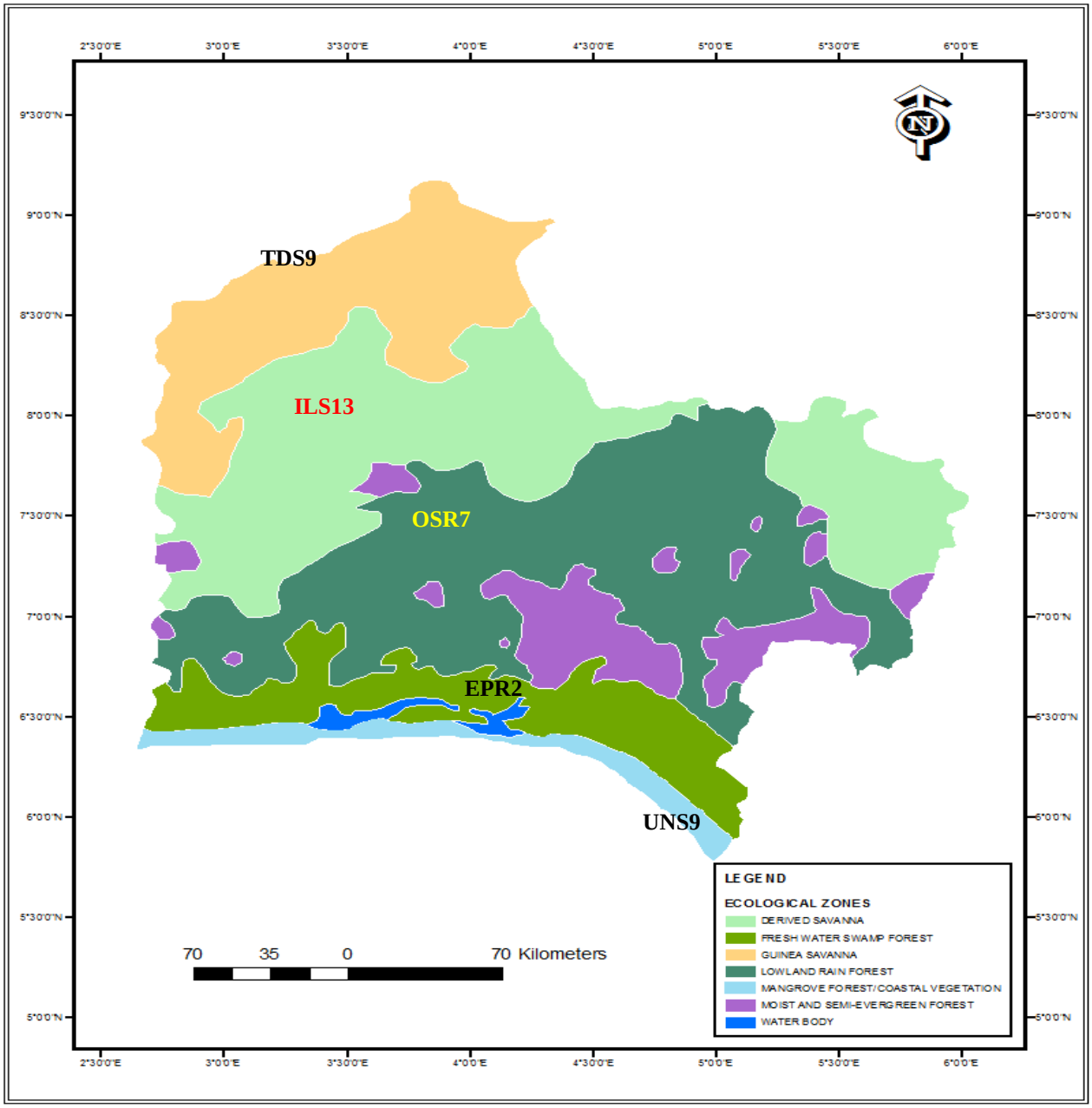


Figure 4.22: Ecological distribution of identified beneficial bacteria isolates

Table 4.33: Identification, similarity match and comparison of beneficial bacteria isolates

Phyto-beneficial bacteria code	(Preliminary identification) Biochemical characterization identity	RDP Similarity match (%)	NCBI Similarity match (%) / Accession Number	Dendogram / phylogenetic similarity match
ILS13	*Bacillus subtilis*	*Bacillus* sp. (100%)	*Bacillus cereus* (99%) / NC 016779.1	*Bacillus niacini*
EPR7	*Bacillus subtilis*	*Bacillus* sp. (100%)	*Flavobacterium psychrophilum* (91%) / NC 009613.1	*Bacillus aeolius*
EPR2	*Bacillus cereus*	*Lysinibacillus* sp. (100%)	*Lysinibacillus spaericus* (99%) / NC 010382.1	*Lysinibacillus boronitolerans*
IGGR11	*Pseudomonas mallei*	*Enterobacter* sp. (93%)	*Enterobacter cloacae* (99%) / NC 016514.1	*Enterobacter pyrinus*
OSR7	*Azomonas insignis*	*Enterobacter* sp. (70%)	*Enterobacter cloacae* (99%) / FP 929040.1	*Enterobacter radicincitans*
IBS8	*Pseudomonas mallei*	*Citrobacter* sp. (97%)	*Klebsiella oxytoca* (99%) / NC 016612.1	*Citrobacter fameri*
UNS9	*Pseudomonas alcaligenes*	*Stenotrophomonas* sp. (100%)	*Stenotrophomonas maltophilia* (99%) / NC 010943.1	*Stenotrophomonas maltophilia*
ABS6	*Pseudomonas mallei*	*Azomonas* sp. (26%)	*Pseudomonas aeruginosa* (96%) / NC 018080.1	*Azomonas macrocytogenes*
TDS9	*Azomonas insignis*	*Myroides* sp. (100%)	-	*Myroides odoratus*
AT-IKS	*Pseudomonas mallei*	*Myroides* sp. (100%)	*Flavobacterium columnare* (90%) / NC 016510.2	*Myroides odoratus*
IPR1	*Azomonas insignis*	*Myroides* sp. (100%)	*Flavobacterium psychrophilum* (93%) / NC 009613.1	*Myroides odoratus*
EPR4	*Pseudomonas mallei*	*Myroides* sp. (100%)	-	*Myroides odoratus*



TDS 9 = *Myroides* sp., ILS13 = *Bacillus* sp., OSR7 = *Enterobacter* sp., EPR2 = *Lysinibacillus* sp., UNS9 = *Stenotrophomonas* sp.

Figure 4.23: Map of Southwestern Nigeria showing ecological location of combined bacteria.

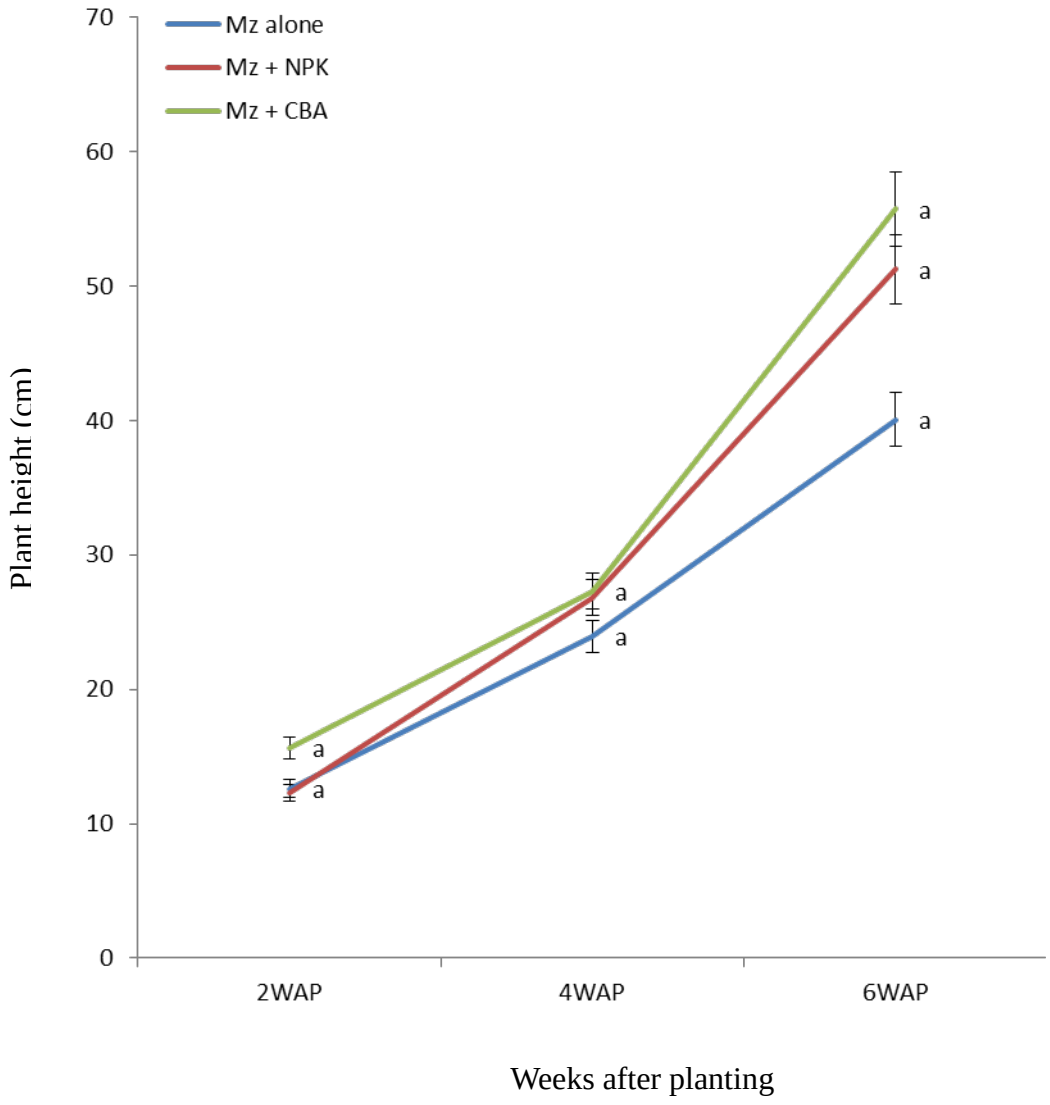


Figure 4.24: Effect of combined phyto-beneficial bacteria on maize plant height

Legend

WAP = Weeks After Planting

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

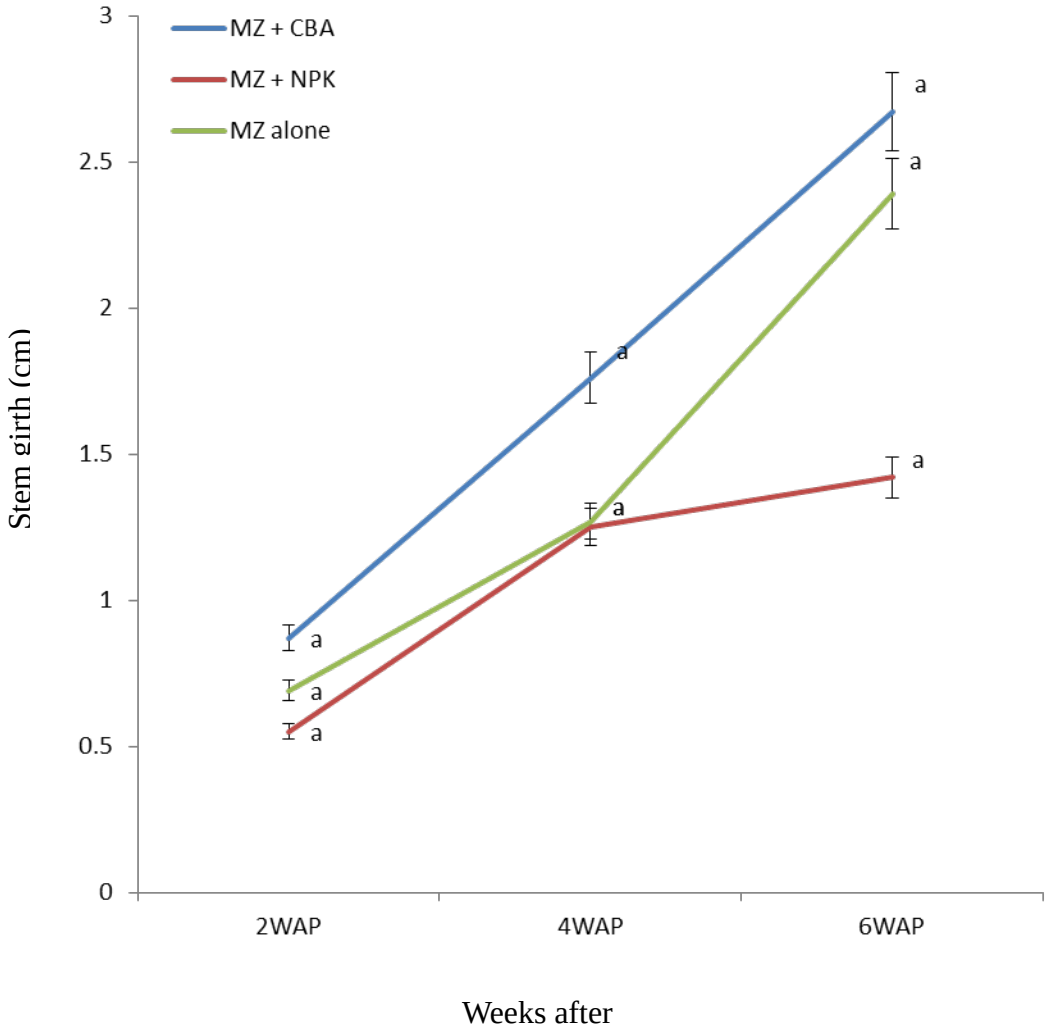


Figure 4.25: Effect of combined phyto-beneficial bacteria on maize stem girth

Legend

WAP = Weeks After Planting

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

High bacteria load was recorded from the rhizosoil of maize plant treated with combined bacteria (MZ + CBA) at the time of harvest, followed by the rhizosoil of untreated maize plant (MZ alone), while the least bacteria load was from rhizosoil of maize plant treated with NPK fertilizer. ANOVA justified that, the bacteria load obtained from the rhizosoil of NPK treated (MZ + NPK) maize plant and that of untreated maize plant (MZ alone) were not significantly ($P < 0.05$) different from each other but were observed to be significantly different from the bacteria load obtained from rhizosoil of maize plant treated with combined bacteria (Figure 4.29).

The pH range was from 6.07 to 6.57. Ironically, the pH of the rhizosoil of untreated maize plant (MZ alone) had the highest pH (6.57) value, followed by the pH of combined bacteria (MZ + CBA) treated soil (6.20), while the least was recorded for rhizosoil of maize plant treated with NPK fertilizer. However, the pH of rhizosoil of untreated maize plant (MZ alone) was significantly ($P < 0.05$) different from other treatments, while the pH of bacteria (MZ + CBA) and NPK treated soils were not significantly ($P < 0.05$) diferent from each other (Figure 4.30).

The treatment significantly ($P < 0.05$) influenced the dry matter of maize plant. There were significant differences ($P < 0.05$) across the treatments. The dry matter of combined bacteria treated maize plant (MZ + CBA) and that of dry matter of NPK treated maize plant were significantly ($P < 0.05$) enhanced far better than that of untreated maize plant (MZ alone). At 6 weeks after planting, the effect of combined bacteria (MZ + CBA) on maize dry matter was significantly ($P < 0.05$) higher and different from that of NPK treated maize plant (MZ +NPK) and untreated maize plant (MZ alone). However, the effect of NPK fertilizer on maize dry matter significantly observed (Figure 4.31).

Data on nitrogen content (mg/pot) present in the rhizosoil after harvest of maize plant are presented in figure 4.32(a). The soil treated with the combined bacteria had the highest nitrogen content compared to that of NPK treated soil, though, they were observed not significantly ($P < 0.05$) different from each other. Ironically, the rhizosoil of untreated maize plant (MZ alone) had nitrogen content higher than that of NPK treated rhizosoil. At harvest, nitrogen uptake (mg/plant) of maize plant treated with combined bacteria (MZ + CBA) and that of NPK (MZ + NPK) were significantly ($P < 0.05$) higher and not significantly different from each other but were observed to be significantly different from that of untreated (MZ alone) maize plant (Figure 4.32(b).

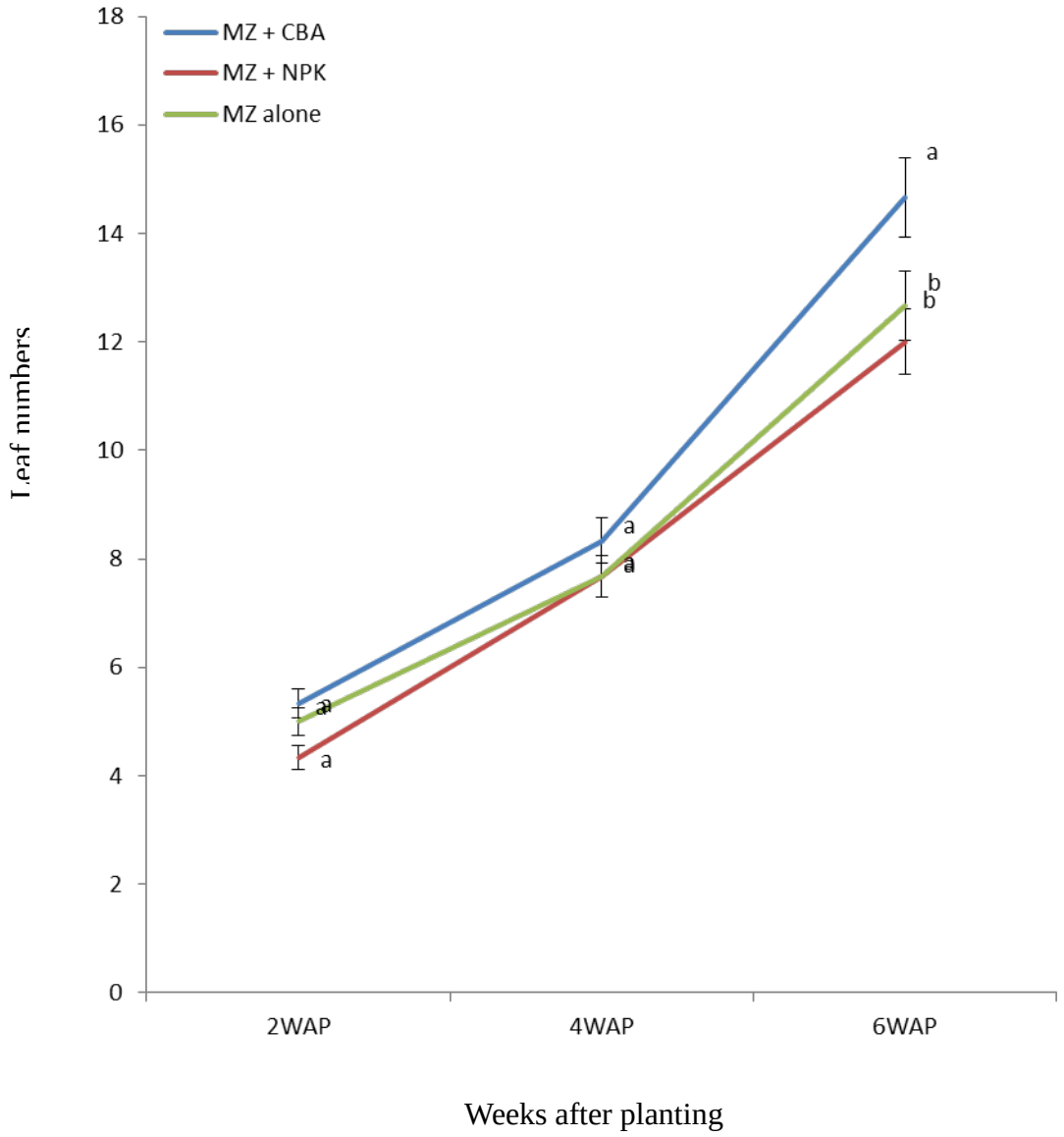


Figure 4.26: Effect of combined phyto-beneficial bacteria on maize plant number of leaves

Legend

WAP = Weeks After Planting

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

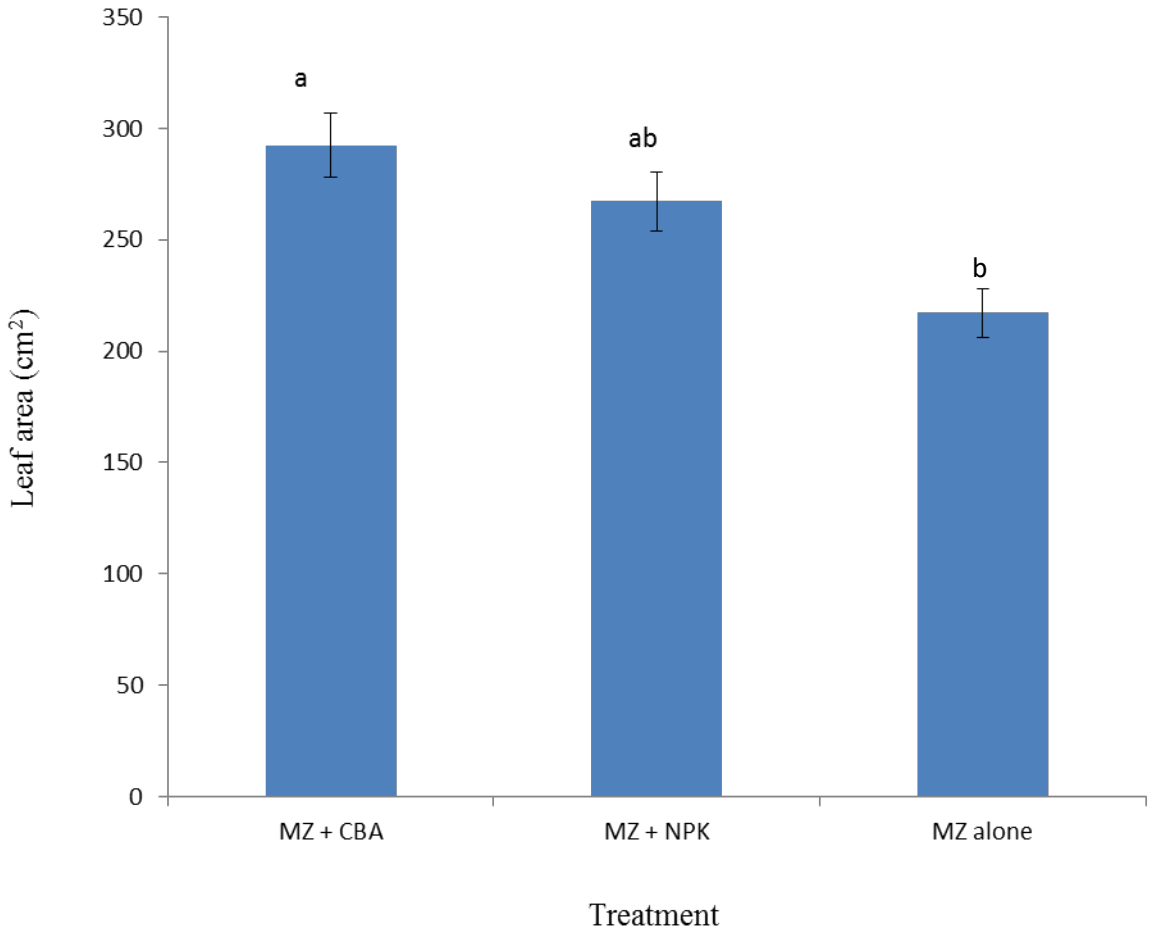


Figure 4.27: Effect of combined phyto-beneficial bacteria on maize leaf area

Legend

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

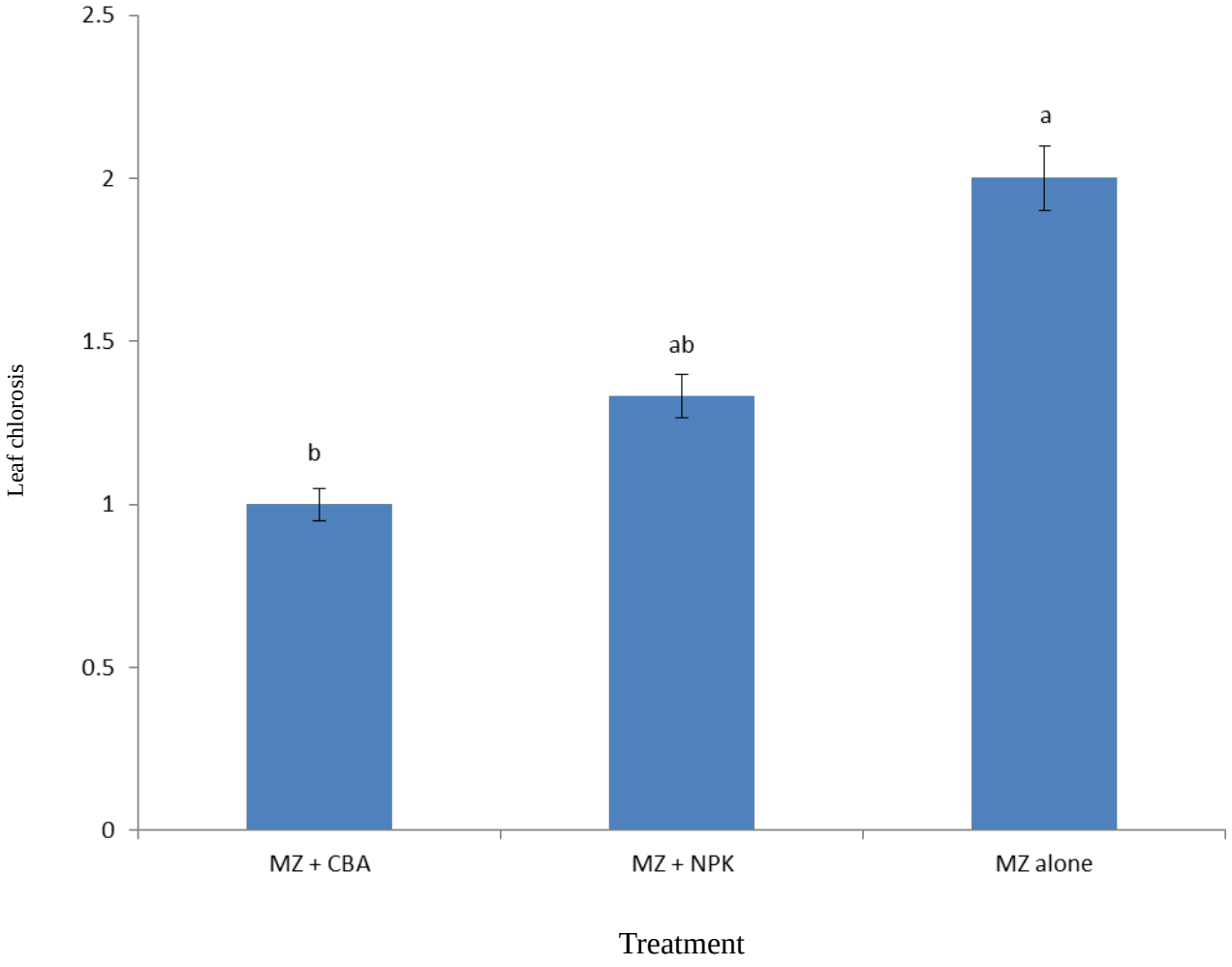


Figure 4.28: Effect of combined phyto-beneficial bacteria on maize leaf chlorosis

Legend

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

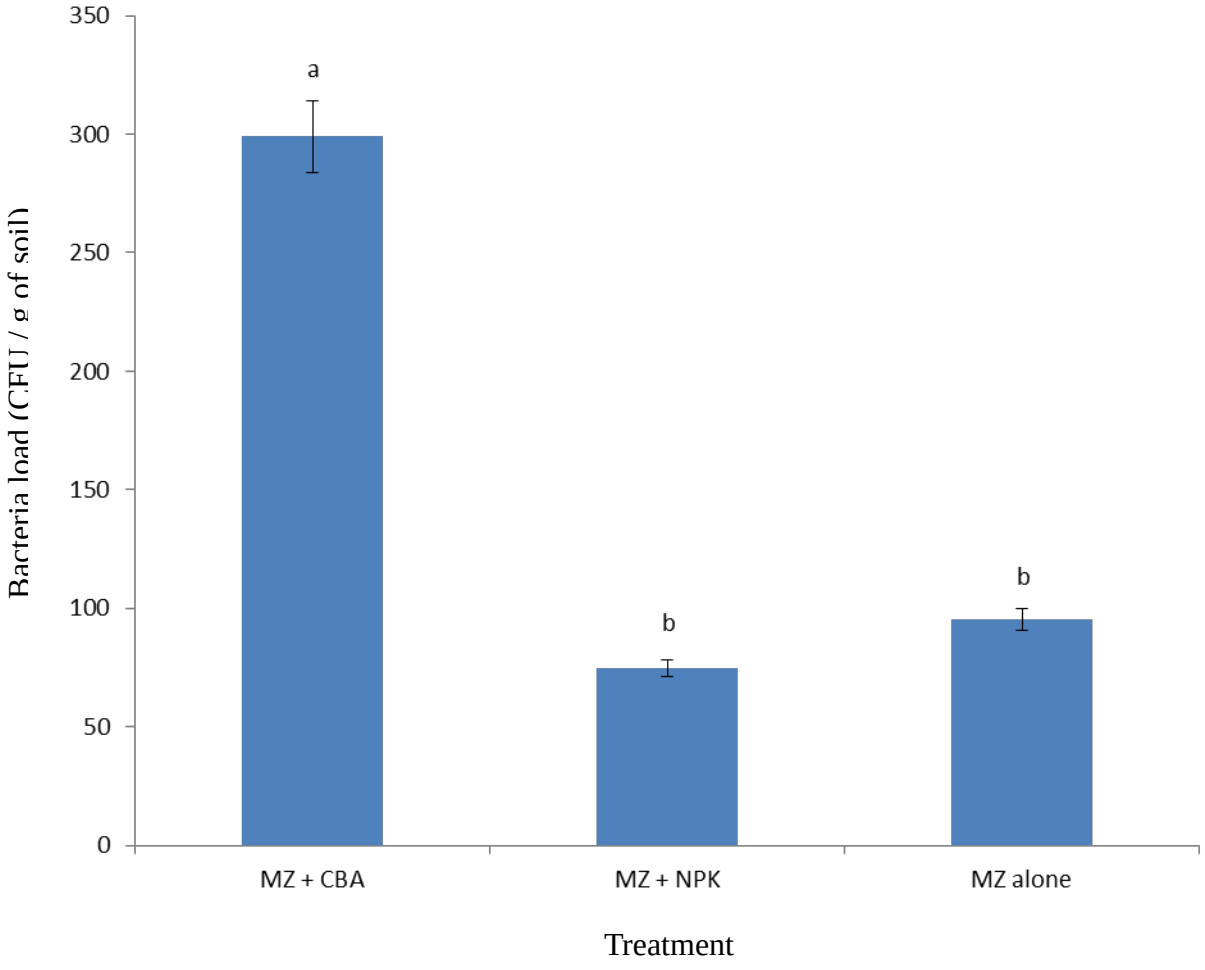


Figure 4.29: Effect of combined phyto-beneficial bacteria on maize plant bacterial load

Legend

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

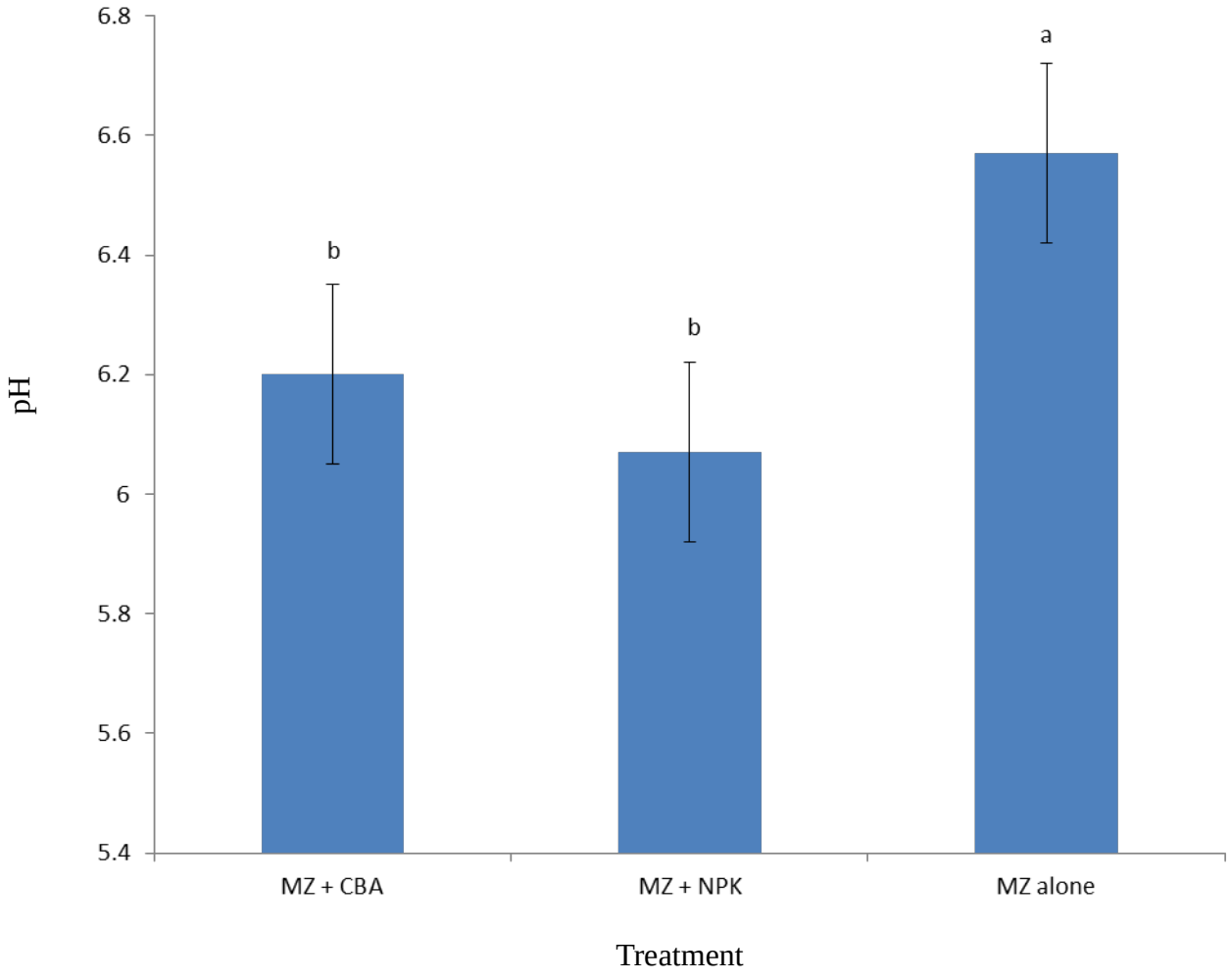


Figure 4.30: Effect of combined phyto-beneficial bacteria on maize plant rhizosoil pH

Legend

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

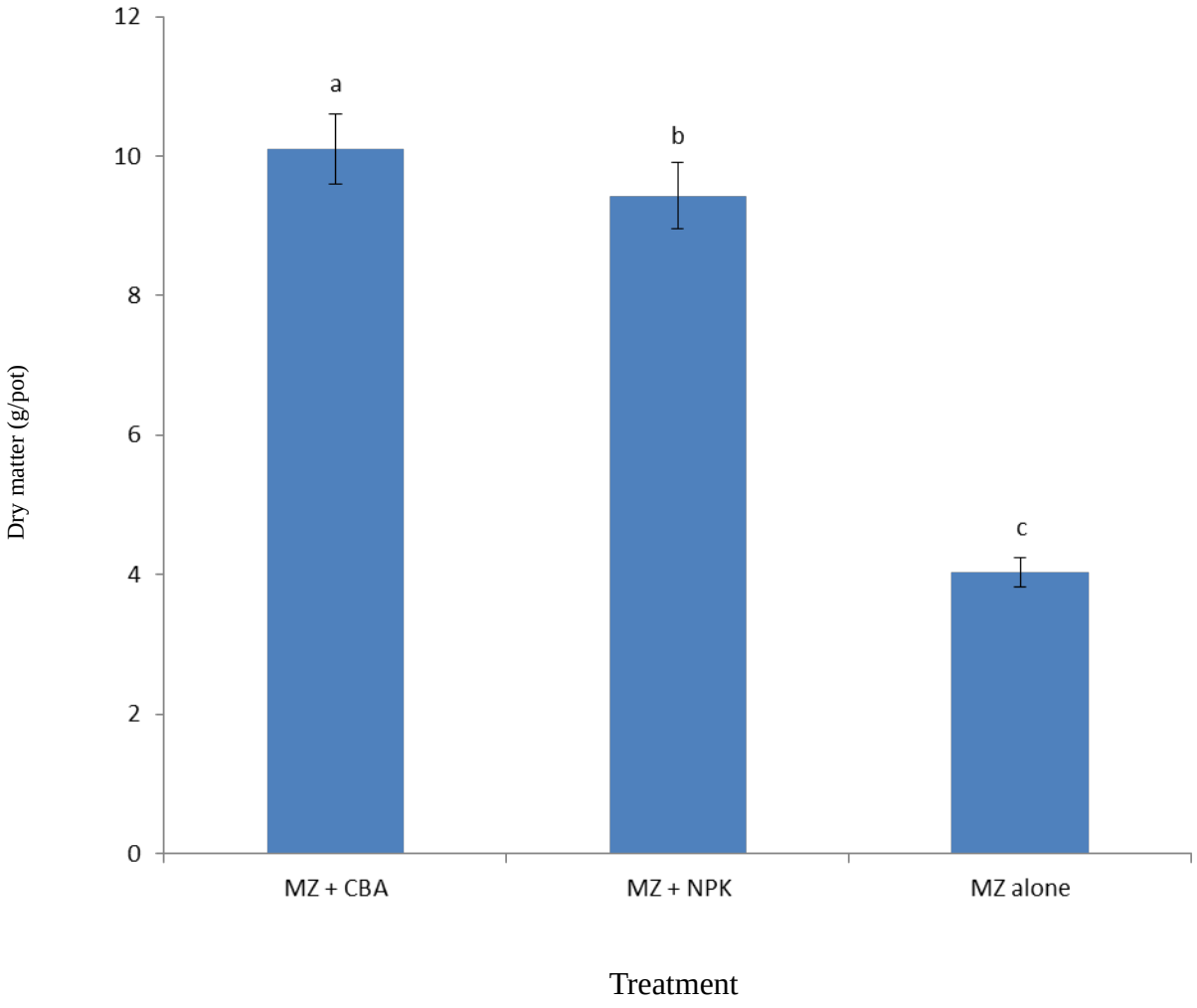


Figure 4.31: Effect of combined phyto-beneficial bacteria on maize plant dry matter yield.

Legend

Mz alone = Maize alone

Mz + NPK = Maize + NPK

Mz + CBA = Maize + Combined Bacteria

The phosphorus content (mg/pot) remained in combined bacteria treated soil (MZ + CBA) was low and not significantly different from that of untreated maize plant (MZ alone). Ironically, NPK fertilized soil had the highest phosphorus content (mg/pot) in the soil (Figure 4.33 (a)). There were significant ($P < 0.05$) differences in phosphorus uptake across the treated maize plant. Combined bacteria application to maize plant significantly enhanced the phosphorus uptake (mg/plant) better than that of NPK treated maize plant (MZ + NPK), while insignificant phosphorus uptake was observed in an untreated (MZ alone) maize plant (Figure 4.33 (b)) at harvest.

The potassium content (mg/pot) in combined bacteria treated soil (MZ + CBA) and that of untreated soil (MZ alone) were not significantly different while low potassium content was recorded for soil of maize plant treated with NPK (Figure 4.34 (a)). Based on the potassium uptake in maize plant (mg/plant), both maize plant treated with bacteria (MZ + CBA) and that of NPK fertilizer (MZ + NPK) significantly and competitively enhanced potassium uptake. Observation also showed that, the combined bacteria (MZ + CBA) and NPK (MZ + NPK) treated maize plant were not significant different in their potassium uptake but were observed to be significantly different from that of untreated maize plant (MZ alone). However, the lowest potassium uptake was observed in an untreated (MZ alone) maize plant (Figure 4.34 (b)). Generally, based on the obtained results, the combined bacteria positively influenced soil quality, thus, significantly enhanced growth and nutrients uptake in maize plant (Figure 4.32, 4.33 and 4.34) at harvest.

The pH of beneficial bacteria isolates in nutrient broth was evaluated at different pH of 3, 5, 7, 9 and 11 so as to correlate it with the pH of the post-experimental soil. Observation showed that, the effect of mean pH significantly varied on growth of beneficial bacteria in nutrient broth. The best pH was 7 and significantly ($P < 0.05$) supported the growth of all the beneficial bacteria better than the other pH values, followed by pH 5 and 3, while pH 9 and 11 were the least. The mean pH (6.18) of collected soil samples (Table 4.2) and that of post-experimental (Sterilized - 6.09, Unsterilized - 6.18) rhizosoil (Table 4.27) were close in value to the best pH (7.0) obtained when nutrient broth was used (Figure 4.35).

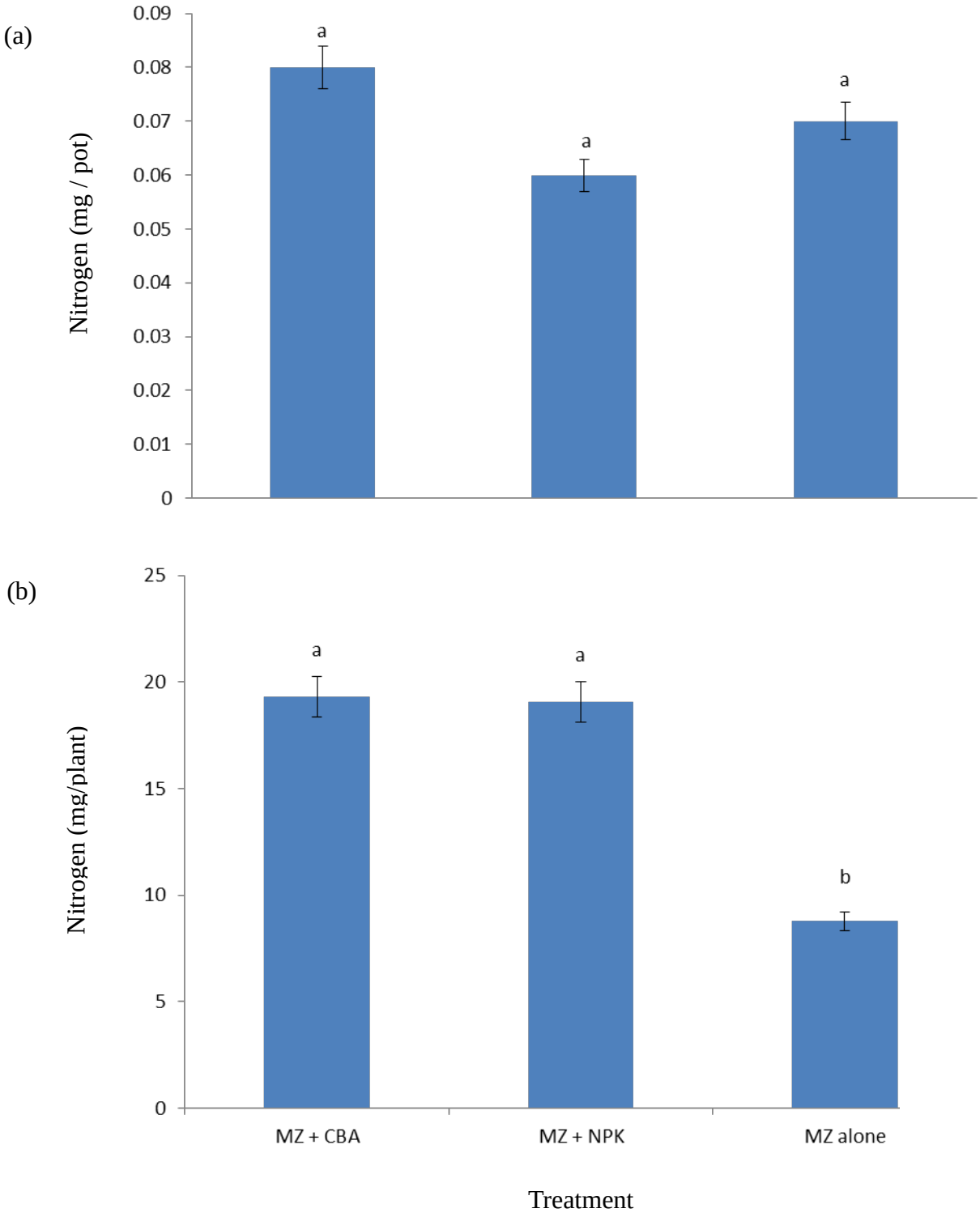


Figure 4.32: Effect of combined phyto-beneficial bacteria on maize nitrogen content.

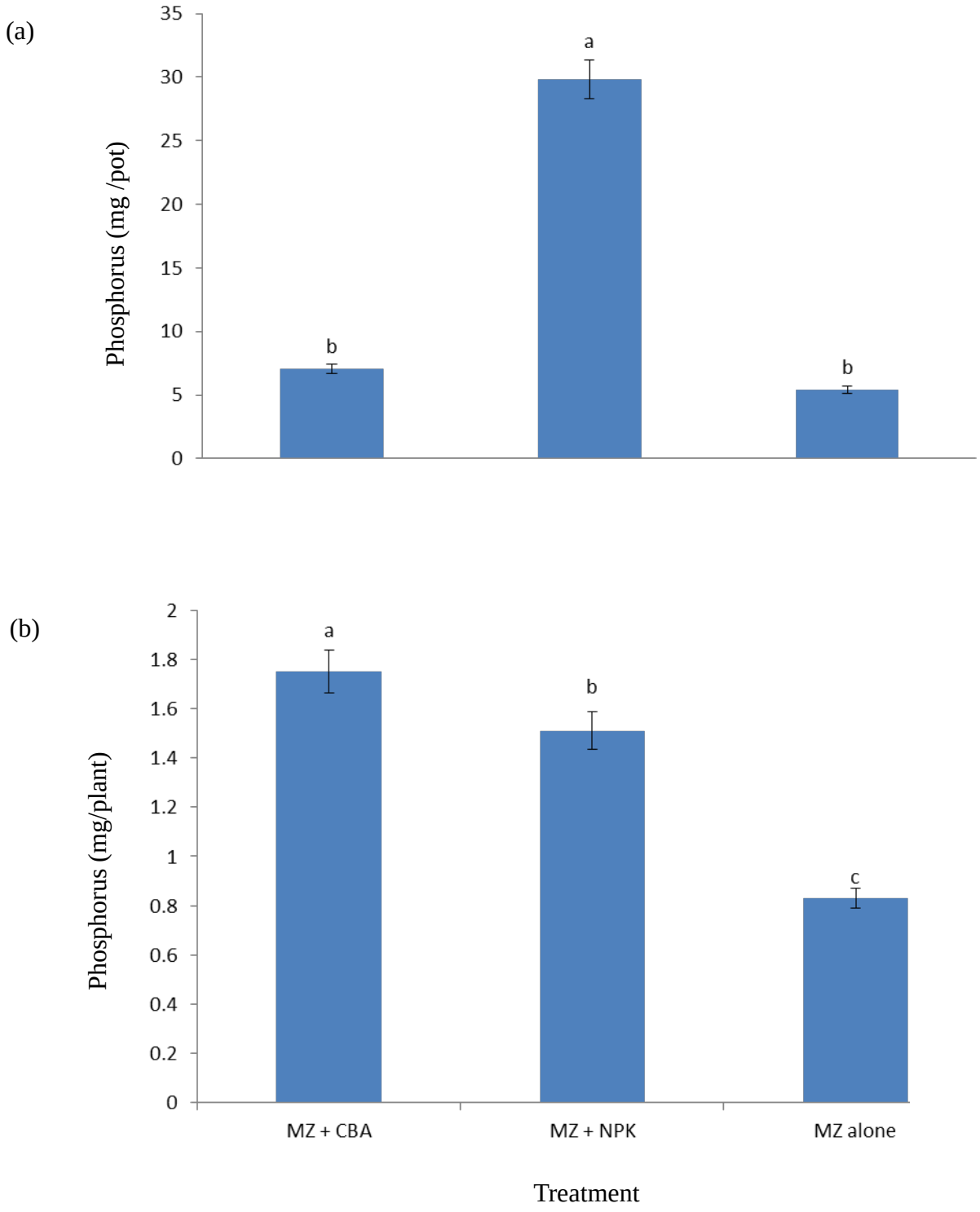


Figure 4.33: Effect of combined phyto-beneficial bacteria on maize plant phosphorus content.

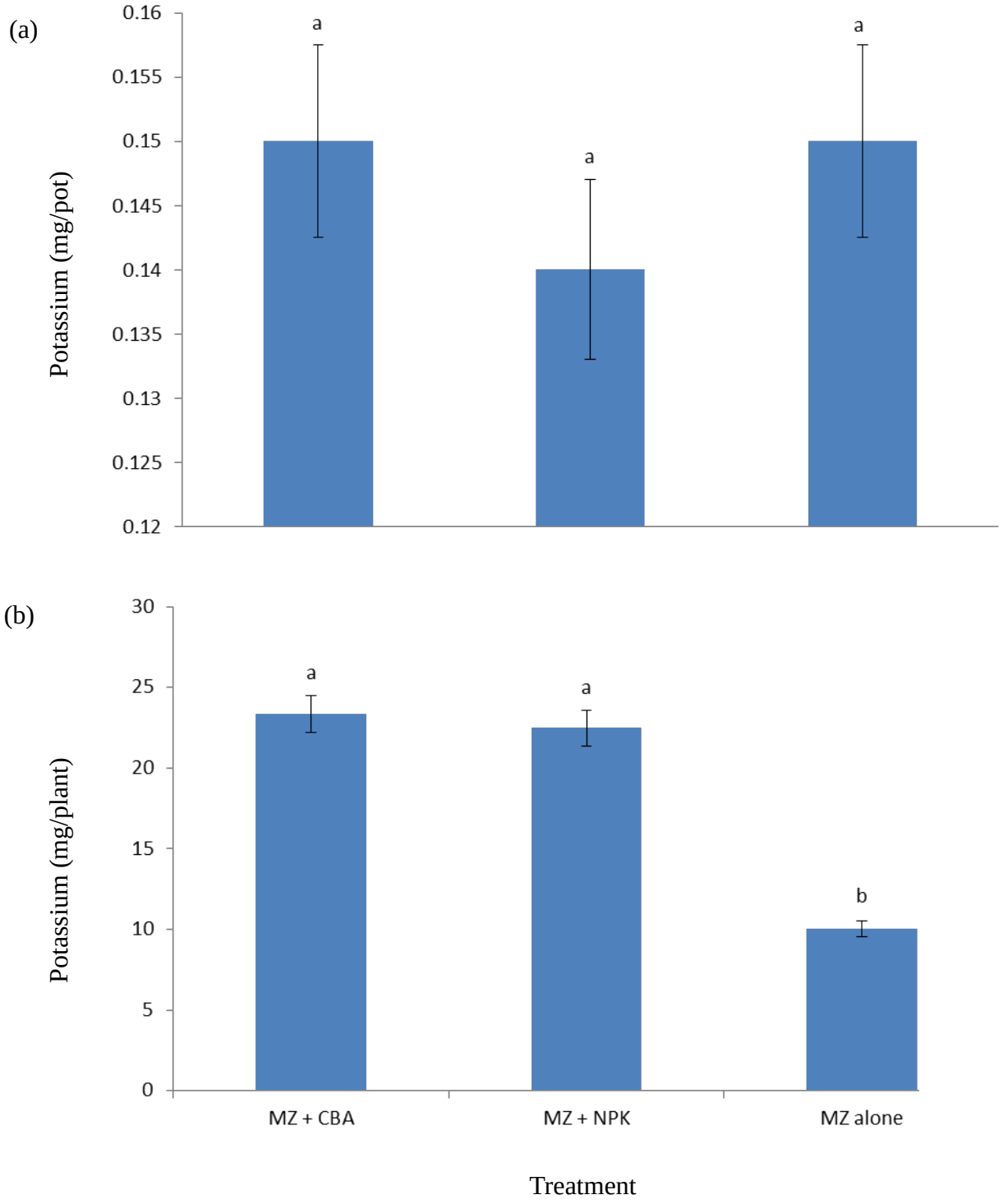


Figure 4.34: Effect of combined phyto-beneficial bacteria on maize plant potassium content.

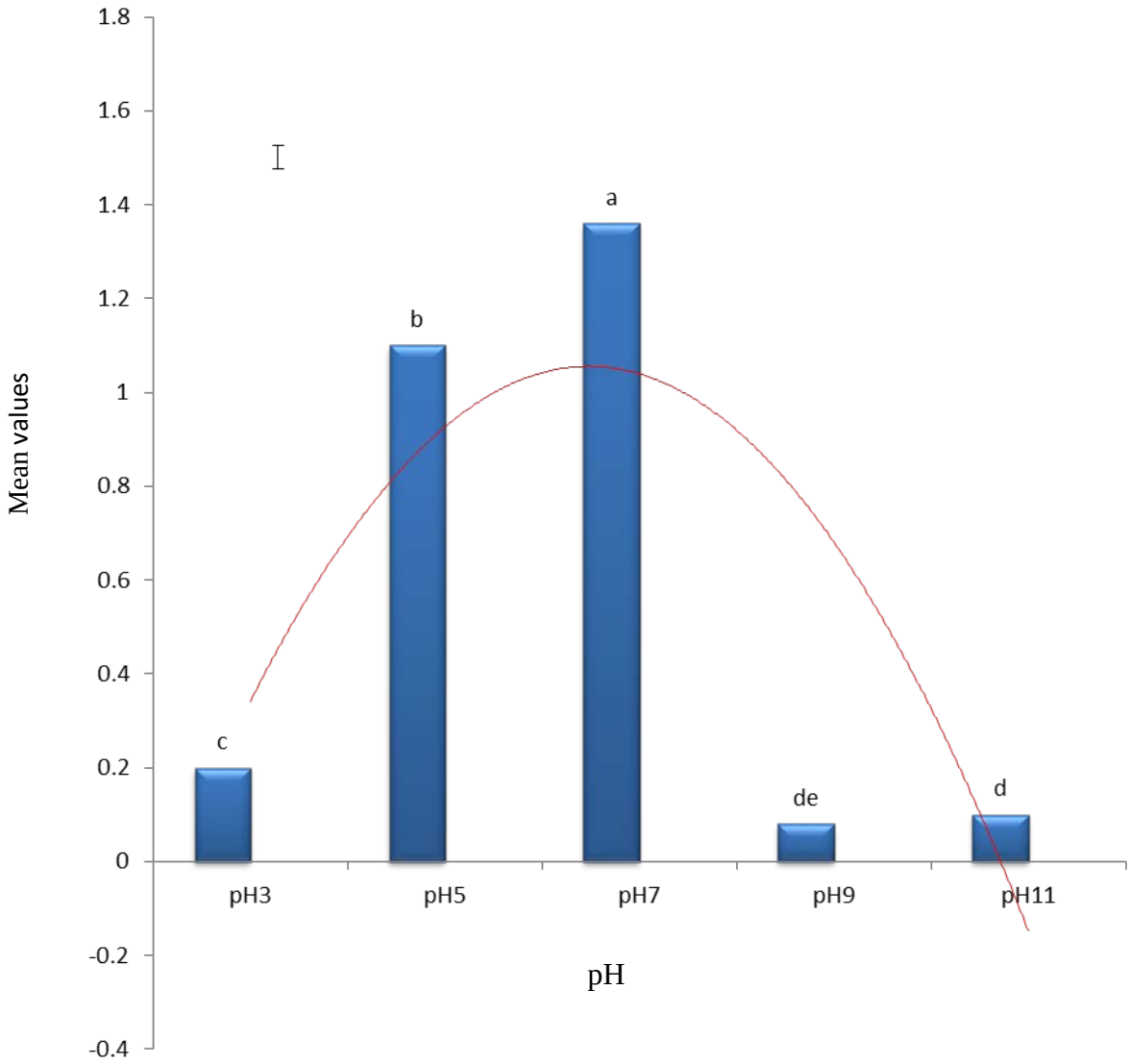


Figure 4.35: Comparative effects of pH on performance of phyto-beneficial bacteria isolates, LSD ($\alpha = 0.05$). LSD = Least Significant Differences.

CHAPTER FIVE

DISCUSSION

Crop production is an important issue which in many ways directs the activities of man. As a result of escalating Africa population there will always be an increasing need to boost the production of important food crops like maize which was researched on in this study. Soil degradation and nutrient depletion have gradually increased and have become serious threats to agricultural productivity in West and Central Africa. Considering, the reduction of fallow from 6 – 2 years has resulted in yield declines from $3tha^{-1}$ to about $0.7tha^{-1}$ for maize (CAB International, 2002).

The field survey information in this study significantly justified the huge variability among the study areas in terms of soils, climatological conditions, organic matter (CAB International, 2002) and maize varieties coupled with well-known problems of farmer's limited access to fertilizers and consequent soil fertility depletion. In Nigeria, high prices of fertilizer cost, on input and transportation from urban to rural / farm areas, import restrictions and scarcity discouraged farmers from using mineral fertilizers (Agboola, 1982). All the farmers in the study areas have been integrated towards the use of plant manure as the cheapest and readily available source of fertilizer as was observed in this study.

The particles size distributions across the study areas of Southwestern Nigeria were mainly sandy – loam (Ayodele and Omotosho, 2008) derived from the crystalline basement complex rocks (Jones and Wild, 1975). The clay content in the study soil was low compared to the silt content, while the sand content was high, thus supporting the observation of Salako (2003). The high percentage of sand obtained in the soil samples collected from maize field in these study areas was closely related to the sand particle level (77.36%) in the soils under maize cultivation obtained by Eludoyin and Wokocha (2011). This could be attributed to erosion caused by the long period of intensive cultivation. With time, if care is not taken in Southwestern Nigeria, soil coarse texture may rapidly be degraded by continuous cropping (Salako, 2003) which may likely lead to extinction of beneficial microorganisms in the soil.

The pH values of the soil samples from the study areas ranged from acidic, neutral to alkaline (Ayodele and Omotosho, 2008), though, this could be ideal for maize growth (Van der Maesen,

1990; Ayodele and Omotosho, 2008). However, few of the soils have acidity problem (pH < 5.0) while 75% have low acidity range of pH 6.0 – 6.9 (Ayodele and Omotosho, 2008) to alkaline (pH 8.0). This suggest that, the variation in the pH of the soil from the study areas could be attributed to the various agricultural and anthropogenic activities within and across the study areas, supported by Kashem and Singh (1998) and Chamon *et al*. (2009). Similarly, continuous cultivation could also cause a significant decline in soil pH. This is even more pronounced when acidifying fertilizers are used (Kang and Balsubramanian, 1990). The variation in the soil moisture content could possibly be due to the application of compost (Hanay *et al*., 2004) and combined application of inorganic and organic fertilizer by maize farmers in these study areas. In addition, the soil of some study areas like Oyo, Ado – Odo, Badagry had low moisture content and were observed to be sandy in nature which may encourage leaching of nutrients through the macropores that abound in such soil types during the rainy season (Salami *et al*., 2003).

Fertile and well managed soils form the basis for efficient crop production (Ayeni, 2011). The soil nutrients in these study areas based on the ecological zones are not sufficient to support maize growth, although, the fact that the addition of mineral fertilizer and organic inputs can be used to correct depletion of soil nutrients and enhance crop productivity in tropical soils has never been in doubt (Amusan *et al*., 2009). Observation from maize field, in Southwestern Nigeria showed that the variation and distribution of soil nutrient elements were not really adequate to enhance maize growth as most of the elements were below the established critical level for soil fertility in Southwestern Nigeria (Agboola and Ayodele, 1985; FMANR, 1990). This could possibly be due to continuous addition of mineral fertilizers such as NPK in different negative formulations (Amusan *et al*., 2009), wrong time, and method of application (Ojeniyi *et al*., 2009) and mismanagement of organic fertilizer.

Previous studies in Nigeria (Salako, 2003; Eludoyin and Wokocha, 2011) attest to the fact that soils in ecological zones of Southwestern Nigeria varied in physical and chemical properties. This could be as a result of range of varying climatic, edaphic, biological and cultural factors, which in turn have resulted into nutrients imbalance. The soil organic matter in the ecological zones are low (Sobulo and Osiname, 1981), thus, the low organic carbon influencing exchangeable cations (K, Ca and Mg), CEC, total nitrogen, P and clay content suggesting the dependence of soil nutrients on organic matter in tropical soils. This agrees with the report of

Adeoye and Mohammed – Saleem, 1990; Salako *et al*. (2002) that the soils of the Savannah region are physically fragile because the topsoil contains a large proportion of sand, causing weak aggregation given the low level of organic matter. Soils of the forest zone were also observed to be low in organic carbon. This could be attributed to continuous rainfall in the LR, FW and MF ecological zones, which has leached out the organic content and indirectly influenced other macro and micronutrients. This agrees with the view of Woodruff (1949) that whenever virgin soils are brought under cultivation and cropping, organic carbon content generally declines because the amount of organic materials returned to the soil decreases sharply and gives chances to erosion and leaching to degrade the soil.

Maize growth would generally perform better in DS compared to other ecological zones. This justified that DS had adequate organic carbon, available P, N, exchangeable cations and extractable micronutrient concentration that can support maize growth. The low soil fertility observed in GS, LR, FW and MG could be related to continuous cropping (Eludoyin and Wokocha, 2011) because the soil is exposed for a long time and left unprotected from damaging climatic influences. The reduced yields in variable lands in the tropics, which become apparent, even after a year or two of cultivation is caused by a lowering of soil fertility and thus poses many constrains on intensive food crop production in tropical Africa (Lal, 1987). This suggest that if adequate care is not taken, soil for arable crop in Southwestern Nigeria will continue to lose its nutrients and would have direct impact on other purposes supporting mankind.

In order to enhance maize growth in Southwestern Nigeria, identifying areas of deficiencies is vital in making soil fertility management recommendations. The low level of fertility of the studied soils will hinder maize production as maize is an aggressive feeder on the soil. It was reported by Adediran and Banjoko (2003) that maize fails to produce good grains in plots without adequate nutrients. Over 70% of the study soil samples were poor in organic carbon which could be attributed to arthropogenic and environmental factors, though, coupled with the fact that the soil of Southwestern Nigeria is fragile in nature (Adeleye and Ayeni, 2009). Similarly, extensive use of chemicals such as fertilizer to improve plant health and productivity as well as for control of pathogens has disturbed the ecological balance of soil which has led to the depletion of nutrients (Sharma *et al*., 2007). This also pointed to the fact that heaps and ridges degrade soil quality, reduce soil chemical and biological qualities of alfisoils located in

Southwestern Nigeria. Although, variation of organic carbon in some study areas could not be ruled out, this would definitely influence both the major and micronutrients. Additionally, high organic carbon was observed in the soil of Igangan (1.90%) and Ibadan (1.84%) study areas, these agreed with the report of Ojeniyi *et al*. (2009) and Jibril and Yahaya (2010) in comparison to the soil nutrients critical level for Southwestern Nigeria (Agboola and Ayodele, 1985; FMANR, 1990).

The low nutrients observed in soil sample from Igboho, Ado- Odo and Ugbo-nla could be attributed to the acidic nature of the soil and high percentage of sand (Baranowski *et al*., 2002). This suggests that, the soils are prone to increased leaching of important component and decreased assimilation of such macroelements as P, K and Mg by plants. The effect of acidifying of soils is visible in decreasing the saturation of exchangeable cations and successive loss of Ca and Mg (Baranowski *et al*., 2002).

Over 70% of the soil samples collected from the study areas including Igboho, Sepeteri, Akure, Ugbo – nla and Aja are deficient in nitrogen content compared with the established 0.15% critical level for soil fertility (Agboola and Ayodele, 1985; FMANR, 1990). Although, maize is a high N demanding crop. This also deduced from the low organic carbon observed in these study areas. Similarly, reports from Sobulo and Osiname (1985) that N deficiency in the soil always shows up when soil contain less than 1% organic matter. The nutrient decline could also be due to nutrient removal while harvesting maize because the crop stores large quantities of nitrogen (Cooke, 1982). Jones and Wild (1975) and FMANR (1990) corroborated that annual dry season burning slash – and – burn land clearing practices cause complete oxidation of litter and humified organic materials with loss of N as oxides into atmosphere.

The high available P within and across the study areas was similar to the report of Eghball and Power (1999). All the maize farmers in the study areas, as observed from the field information obtained in this study, make use of plant manure which may have invariably contributed to the increase in available P, thus agreeing with the report of Zheljazkov and Warman (2004); Gharib *et al*. (2008). The extremely high available P observed in Ilora soil sample could be as a result of consistent use of poultry manure which was confirmed by Salako (2008) that poultry manure improved surface P and maize grain yield significantly. This study, therefore, reconfirms the report of Adeleye and Ayeni (2009) and Ayeni (2011) that poultry manure may likely enhance

soil P. Moreso, Ayeni (2010) have actually demonstrated the use of animal manures and agrowastes for soil fertility maintenance, but the claim that they are low in nutrient quality and bulky was contrary to the high nutritional soil quality most especially available P reported for Ilora and Badagry study areas where poultry manure has always being the source of organic fertilizer to local farmers to enhance maize growth. This is in agreement with the report of Agwu (2006) and Ewulo (2005) that animal manures increased soil nutrients.

With the exception of K and Ca that are moderately high (Ayodele and Omotosho, 2008; Eludoyin and Wokocha, 2011), other exchangeable cations (Mg, Na and ECEC) significantly varied in low to medium quantity across the study areas based on the soil nutrients critical level for Southwestern Nigeria (Agboola and Ayodele, 1985; FMANR, 1990). Even though, K and Ca were observed to be dominant among the exchangeable cations but their significance may not be observed as the low content of Mg, Na and ECEC may limit maize growth, reflecting the high degree of weathering and leaching associated with soil forming processes (Kang *et al*., 1991).

In comparison with the soil nutrients critical level (Agboola and Ayodele, 1985; FMANR, 1990), the extractable micronutrients (Zn, Cu, Mn and Fe) are within the sufficient range across the study areas. Over 80% of the soil samples in the study areas showed that, the extractable nutrients are adequate enough far beyond the observation reported by Ayodele and Omotosho (2008). This suggests that, maize farmers in Southwestern Nigeria may have started adopting soil fertility management strategies to enhance food crops.

There are evidence that, maize is a staple and popular food for the populace of Nigeria (Ayeni, 1991; Iken and Amusa, 2004) and mostly intercropped with crops like cassava, vegetables and yam to mention a few in peasants farming due to the decrease in virgin land (Dalal and Mayer, 1986a; Aweto *et al*., 1992). Therefore, nutrients depletion observed in soils of maize field in Southwestern Nigeria may as well be associated with the pre – history of continuous intercropping of cassava and maize in most of the study areas. This findings re – affirm the report of Aweto *et al*. (1992) that continuous intercropping of cassava and maize appeared to have a greater degrading effect on soil in terms of soil organic, total nitrogen and available phosphorus while Dalal and Mayer (1986a) also reported that soil C and N were greatly reduced in the cereal belt of Southern Queensland following land clearing. Further physical and chemical

degradation of soil in maize fields of Southwestern Nigeria will not only support food insecurity but it will also complicate and disorganize soil structural ecosystem beyond restoration.

Despite the acknowledged importance of soil and rhizosphere bacteria in Southwestern Nigeria, little is known about the variation of bacteria load associated with maize plant rhizosphere in Southwestern Nigeria. This study justified that, bacteria population compared favourably with most agricultural soils as reported by Paul and Clark (1996); Gomes *et al*. (2001); Orole and Adejumo (2011). Studies have shown that microbial load changes with location (Barreto *et al*., 2008), host specificity, geographical distribution, plant age, and tissue type (Kobayashi and Palumbo, 2000). This is in agreement with the result obtained in this study, because, there were variation in bacteria load within and across the study areas. Several factors may be responsible for the variation in the bacteria load. It was pointed out that nutritional status of the maize varieties, soil structure, micronutrient status of the soil, root morphology and physiology caused by diurnal variations, root ageing and root emergence (Sullivan, 2004) may have directly or indirectly influence the variation observed in bacteria load in this study. Based on the source of collection, the bacteria load accounted for higher percentage in the bulk soil than the rhizosphere. This agreed with actinomycetes population reported by Barreto *et al*. (2008) but contrary to the report of Raghvendra and Harbans (2008) that rhizospheric population *Azotobacter chroococcum* was always higher than non- rhizospheric soil, at all stages of plant growth.

For many years, *Fusarium verticillioides* has been a major patho – toxigenic fungi, known to cause seedling blight, seed and stem rot and produce toxic metabolites, thus threatens maize growth in Nigeria (Adejumo *et al*. 2007). The use of pesticides to control fungal pathogens is usually unsuccessfully, moreso, pesticides pose health hazards to human health and the environment. The increasing cost of pesticides, particularly in the Sub – Saharan Africa, and consumer demand for pesticides – free food across the globe has led to a search for substitutes for these products (Gerhardson, 2002). Evaluation of antagonistic activity of soil bacteria successfully controls the pathogenic fungi (*Fusarium verticillioides*) in – vitro (Cook *et al*., 1995; Berg *et al*., 2002; Odebode, 2006; Abiala *et al*., 2010) and in – vivo (Raupach and Kloepper, 1998; Ramamoorthy *et al*., 2002). Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture (Postma *et al*., 2003).

The result in these study justified the report of several workers (Ramos *et al*., 2000; Liasu and Shosanya, 2007; Vega, 2007) that rhizosphere of plant have high percentage of antagonistic bacteria compared to their counterpart soil. This could probably be due to rhizosphere effect (Morgan and Whipps, 2001) to harbor greater microbial population, suggesting fierce competition for nutrients as well as the existence of species which show a variety of functional diversity and metabolic versatility (Dube and Yeole, 1999; Sinha *et al*., 2001).

Antagonistic bacteria isolates were not recorded for Sepeteri, Akure, Ado – Odo, Abigi and Aja. This may be as a result of soil quality because the field survey data and the physico- chemical analyses in these study areas showed that, the soils were of low quality, thus, moderately deficient to support maize growth, which could as well contribute to low diversity of beneficial and antagonistic rhizospheric bacteria. Similarly, it is commonly assumed that decline in the biodiversity of the soil biota may be due to intensified agricultural practices and may reduce the essential ecosystem functions as well as the ability of agricultural system to withstand periods of stress (McCaig *et al*., 1999; Buckley and Schmidt, 2003).

Remarkable observation in this study showed that, DS ecological zone had the best soil physic – chemical properties and the highest antagonistic rhizospheric bacteria. This indicates that, diversity and community structure in the rhizosphere is influenced by both plant and soil type (Latour *et al*. 1996). Likewise, non – leguminous crops do select specific bacterial groups in the rhizosphere (Josserand *et al*., 1995).

Phosphorus solubilization is one of the plant growth promoting characteristics that promote yield because they enhance both physiological and biological activities in plants. Over 45% of the collected soil samples in this study are deficient of available P (Gaind *et al*., 2000). Generally, deficiency of P is a serious threat to maize growth (Ayodele and Omotosho, 2008) in Southwestern Nigeria. Covering up the deficiencies of P, more than 64% of the bacteria isolates in this study solubilized Calcium phosphate (Gaind *et al*., 2000) which is likely to facilitate their use as reliable components in the management of sustainable agriculture (Zaidi *et al*., 2009) and maize growth, though, variation was observed in their solubilization potentials.

Similarly, plant growth promoting characteristics of many soil bacteria may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and

other plant growth promoting activities (Arshad and Frankenberger, 1993; Glick, 1995). It has been estimated that 80% of bacteria isolated from the rhizosphere can produce plant growth regulator IAA (Patten and Glick, 1996). Consistent with the report of Joseph *et al*. (2007), variation was observed in the amount of IAA produced by the bacteria isolates (Jain and Patriquin, 1985; Glick, 1995) coupled with the fact that many rhizobacteria has potentials to produce IAA. The remarkable observation in this study was that, all the test bacteria were able to utilize L-tryptophan and D-L tryptophan. Many research (Oyekanmi *et al*., 2008; Abiala *et al*., 2010; Killani *et al*., 2011) works has justified various functions of beneficial soil bacteria in Southwestern Nigeria but their potentials to produce IAA has been relegated to the background. There may have been report on utilization of L-tryptophan by soil bacteria to produce IAA, but based on findings in this study, soil bacteria utilized D-L tryptophan and the quantity of IAA produced was far higher than that of L-tryptophan.

Chitinase activity of the bacteria isolates in this study agreed to that of Barreto *et al*. (2008) for actinomycetes isolates, likewise, not different from the chitinase activity in plate assays reported from the bacteria isolates of Berg *et al*. (2002). Interestingly, the potentials of bacteria isolates chitinase activity, IAA and phosphate solubilization did not differ in this in – vitro study. Report has established that, results of in – vitro experiment and in – vivo activity may at times not correlated (Wong and Baker, 1984; Vessey, 2003), these in –vitro assays indicated the potentials of the isolates being tested for applications such as biological control, composting, and plant growth promotion, as was also reported by Cavaglieri *et al* (2004).

Improvement of seed germination parameters by rhizobacteria has been reported in other cereals such as sorghum (Raju *et al*., 1999) and pearl millet (Niranjan *et al*., 2003). The bacteria isolates under laboratory condition improved seed germination beyond the control, as 100% seed germination was achieved. This conform with the report of Shaukat *et al*. (2006a); (2006b) where it was observed that some plant growth promoting rhizobacteria induced increases in seed emergence, in some cases achieving increases up to 100% greater than controls. These findings may be due to the increased syntheses of hormones like IAA examined in this study, which could have triggered the activity of specific enzymes that promoted early germination, such as amylase, which may have brought an increase in availability of starch assimilation (Gholami *et al.* 2009).

Enhancement of plumule and radicle length by bacteria isolates may be due to release of plant growth promoting substances (Sharma *et al*. 2007). This report, thus, agreed with the report of Gupta *et al*. (1998); Joseph *et al*. (2007) that some tested rhizobacteria isolates could have exhibited more than two or three plant growth promoting traits, which may promote plant growth directly or indirectly or synergistically. Moreso, the positive effect of *Azospirillum* and *Pseudomonas* on growth parameters of cereals (Bashan *et al*., 2004) is as well not different from what is obtainable in this study on the effects of bacteria isolate on maize growth.

All the rhizobacteria isolates that exhibited plant growth promoting characteristics beyond the control could be reffered to as beneficial rhizobacteria. Management of phytopathogenic fungi provides an environmentally safe approach that does not pose any danger to health. The implication of this in biological control and crop improvement as far as end users are concerned is wonderful because the harvested crops will not contain unacceptable residues. In the course of antagonist – pathogen interaction in this study, inoculation and bacterization of maize seed sowed directly into the soil (Hayat *et al*., 2010), showed positive effects on maize plant heigth, stem girth, number of leaves and leaf area as was also reported by Oyekanmi *et al*. (2008). The bacteria load varied (Gomes *et al*., 2001) probably as a result of variation in the soil pH (Arslan *et al*., 2008) of treatments, thus accounted for the variation observed in the maize plant heigth, stem girth, number of leaves and leaf area (Joseph *et al*., 2007; Killani, 2010) which is even better than the controls.

Considerable researches have been done to investigate antagonistic microbes for use in seed treatments as reported by Callan *et al*. (1990) and Baird *et al*. (1994). Over 95% of the rhizobacteria isolates antagonize *Fusarium verticillioides* in the screenhouse experiment. Remarkable is the high antifungal activity exhibited as disease expression was delayed between day 14 and day 21. This suggested that, the rhizobacteria takes sometimes to exhibit their antifungal activity probably due to the report of Duffy and Defago (1999) and Bloemberg and Lugtenberg (2001) that, the production of antifungal metabolites is subject to complex regulation, allowing bacteria to sense their own population density and to respond to different environmental factors which could be a better picture of root colonization and dynamics of bacterial rhizosphere communities (Berg *et al*. 2002). Likewise, this could be attributed to the fact that, the rhizobacteria isolated in this study are not early colonizers as was against the

speculation of Whipps and Lumsden (2001); McLean *et al.* (2004). Hence, this is a promising field for future research, for more soil samples to be collected periodically in Southwestern Nigeria in – view to isolates early colonizing plant growth promoting rhizobacteria (Lambert *et al*., 1987; Lalande *et al*., 1989; Cattelan *et al*., 1999). However, this study does not include mechanism of biocontrol but agreed that antibiosis, known as one of the biocontrol mechanisms must have played a vital role in this present study.

Based on the laboratory and the pre – screenhouse results, correlation of agronomical and pathological data's could be a useful and rapid selection criteria for plant growth promoting rhizobacteria. There was strong positive correlation between radicle and plumule length in the laboratory assay which is part of the data's expected to predict the agronomical activities of maize plant in the screenhouse. Similarly, strong positive correlation was also observed between the pathological parameters, judging by disease expression at day 14 and 21, this, indicates that some bacteria isolates were not able to control the pathogenic fungi which eventually led to expression of diseases on maize plant. Bacteria load negatively correlated with disease expression, that is, the lower the antagonistic bacteria load in maize rhizosphere, the more chances for disease to be expressed. Similarly, the higher the antagonistic bacteria load in maize rhizosphere, the less chances for disease expression on maize plant.

Furthermore, maize seed germination negatively correlated with disease expression, indicating that, delay in maize seed germination may likely encourage disease expression, thus, influences maize seedling morphogenesis. This could be attributed to the fact that, the fungal pathogen colonizes the maize seed ahead of the antagonistic bacteria. Similarly, early maize seed germination may likely discourage disease expression in maize plant. This conforms to the report of Muhammad and Amusa (2003) that the great reduction of pathogen population densities in the rhizosphere soil could be as a result of lower proliferation rate of the pathogen in the rhizosphere already colonized by the antagonist. Therefore, based on these results and the potential implications for high – throughput screening, exactly 19 out of 48 isolates were subjected to biochemical characterization as plant promoting rhizobacteria.

In this study, a relatively large population of the rhizobacteria is dominated by Gram negative forms (EPR4 - *Pseudomonas mallei,* ABS6 - *Pseudomonas mallei,* TDS9 - *Azomonas insignis* IGBR11 - *Azomonas insignis,* ADS14 - *Pseudomonas pseudomallei,* IBS8 - *Pseudomonas*

mallei, OSR7 - *Azomonas insignis,* EPR3 - *Xanthomonas fragariae,* AT-SKR - *Azomonas insignis,* IPR1 - *Azomonas insignis,* AT-IKS - *Pseudomonas mallei,* AKR5 - *Xanthomonas ampelina,* AT-ILR - *Azomonas macrocytogenes,* IGGR11 - *Pseudomonas mallei,* UNS9 - *Pseudomonas alcaligenes*) that exhibit various functions. This is attributed to the liability of plant growth promoting rhizobacteria to release IAA, synthesize ACC deaminase, lower ethylene level, secrete siderophones and antibiosis, release volatiles, form hydrolytic enzymes, solubilize phosphorus, fix nitrogen and in – voke induced systemic resistance (Glick, 1995). Isolates that are capable of performing these functions are termed beneficial (Johri *et al*. 2003). Additionally, Gram positive bacteria (EPR2 - *Bacillus cereus,* EBS8 - *Bacillus subtilis,* ILS13 - *Bacillus subtilis,* EPR7- *Bacillus subtilis*) were also isolated in this study as they were observed to have exhibited high degree of plant growth promoting characteristics. Consequence to this study, it is pertinent to consider the possible distribution of pathogenic / potential pathogens within the bacteria isolates, apart from their plant growth promontory activities.

The rhizosphere, considered to be a hot spot of bacterial diversity, harbours bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favour plant growth. This is in turn beneficial to the whole rhizsophere microbiota through the highly nutritive and energetically rhizodepositions (Aragno, 2005). A continued exploration of the natural biodiversity of soil microorganisms and the optimization and manipulation of microbial interactions in the rhizosphere of crops represents a prerequisite step to (Rawat *et al*., 2011) a well defined and rapid selection of useful bacterial isolates carried out in this study, in view to develop more efficient biofertlizer for sustainable maize growth in Southwestern Nigeria.

All the beneficial rhizobacteria that exhibited plant growth promoting characteristics individually gave better plant height. The height of plant is an important growth characteristics directly linked with the productive potential of plants in terms of grains. An optimum plant height is claimed to be positively correlated with productivity (Saeed *et al*., 2001).

Enterobacter (OSR7) increased stem girth of maize more compared to the controls and other bacterial isolates. This agreed with the report of of Aragno (2005), that, *Enterobacter* is among the beneficial bacteria that play significant role in plant growth. Similarly, *Enterobacter* may

possess other beneficial potentials to retain appreciable amount of assimilates in the stem for leaf production (Law – Ogbomo and Law – Ogbomo, 2009).

Number of leaves differed with respect to the beneficial bacteria isolates. This may have been enhanced by the differences in the plant growth promoting characteristics of the isolates. The performance of *Myroides* (EPR4), *Bacillus* (EPR7), *Myroides* (TDS9) and *Myroides* (AT – IKS) agreed with Oyekanmi *et al.*, (2008) and Gharib *et al.*, (2008) that beneficial microorganisms has potentials to enhance plant number of leaves. As regards the leaf area, all the beneficial bacteria competed with NPK fertilizer treated maize plant. Surprisingly, the NPK fertilizer treated maize plant had the highest leaf area. This may have been partly due to nutrient availability which must have been enhanced and probably favoured by environmental factors.

Furthermore, the bacteria isolate increased plant height, stem girth, number of leaves and leaf area. This is in-line with an increase observed in different crops inoculated with *Pseudomonas, Azospirillum* and *Azotobacter* strains (Shaukat *et al*., 2006a; Shaukat *et al*., 2006b). Plant growth promoting rhizobacteria may enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants, decreasing heavy metal toxicity in the plants and antagonizing plant pathogens as reported by Burd *et al.* (2000). These may be responsible to the significant growth in maize morphological parameters reported in this study. However, cases where plant height, stem girth, number of leaves, leaf area were low, coupled with appearance and disappearance of leaf chlorosis and expression of leafspot diseases in an unsterile soil, may be accounted for differences in colonization which may also be due to changes in both the nutrient content of the soil and microbial activity (Jansa *et al*., 2003; Aragno, 2005).

There is no doubt that, nutrient availability is limiting maize growth in Southwestern Nigeria based on the chemical content analysis carried out on the collected soil samples from the study areas. This study hypothesized that, beneficial bacteria play significant roles in nutrient uptake as well as soil fertility as documented by other authors (Johri *et al*., 2003). This hypothesis was proofed in this study on bacteria isolates indigenous to Nigeria. The potentials of bacteria isolates to enhance nutrient uptake (nitrogen, phosphorus and potassium) were evaluated on maize. Based on these findings, nutrient uptake varied among the tested bacteria isolates which indirectly influence maize growth. Among the dominant and tested bacteria isolates that enhance

nutrient uptake in maize are; *Myroides*, *Enterobacter*, *Citrobacter*, *Bacillus*, *Lysinibacillus*, *Stenotrophomonas* and *Azomonas*. *Enterobacter*, *Bacillus*, *Stenotrophomonas* and *Citrobacter* has been successfully documented to enhance nutrient uptake and plant growth (Lambert and Joos, 1989; Biswas *et al*., 2001; Verma *et al*., 2001; Asghar *et al*., 2002; Bashan *et al*., 2004; Tilak *et al*., 2005; Vega, 2007; Gholami *et al*., 2009; Hayat, 2010). Report on rhizospheric *Myroides* and *Lysinibacillus* to enhance maize growth and nutrient uptake in Southwestern Nigeria is currently scarce, therefore, with continuous and further research, their potentials as beneficial rhizobacteria for sustainable maize growth was reported in this study. Both *Myroides* and *Lysinibacillus* enhanced maize growth and nutrient uptake far better than other tested bacteria isolates. The low content of N, P and K observed in negative control (Maize alone) and positive control (Maize + NPK) could be attributed to the absence of beneficial bacteria to enhance nutrient uptake, thus, re – affirm that beneficial bacteria alongside with other factors (fertility of native soil, application of chemical fertilizers, the growth stage of the plant and environmental condition) as mention by Ologunde (1974) are needed to enhance nutrient uptake in maize.

Inoculation of maize seed with beneficial bacteria at the point of sowing resulted in positive impact on biomass production, mineral enhancement uptake and transfer of nitrogen to the plant. Moreover, soil microorganisms that colonize the rhizosphere assist plants in the uptake of several vital nutrients, such as N, P and K from soil (Cocking, 2003). Nitrogen is required by maize plant in high quantity when compared to P and K. Enhancemet of maize growth, shoot dry weight and N uptake by *Enterobacter* isolates is remarkable, thus, suggest evolutionary niche and relationship with maize plants in Southwestern Nigeria.

Soils of Southwestern Nigeria are not really deficient in phosphorus based on the data obtained in this study but solubilization and uptake for maize growth is the major challenge, which often cause P – deficiency and in – turn, restrict maize. Soil microorganisms are involved in a range of processes that affect P transformation and thus influence the subsequent availability of P (as phosphorus) to plants roots (Richardson, 2001). Most of the beneficial bacteria enhance P uptake, particularly active are those that belong to the genera *Enterobacter* and *Bacillus* (Chung *et al*., 2005; Whitelaw, 2000), *Stenotrophomonas* (Ryan *et al*., 2009) and *Citrobacter* (Thaller *et al*., 1995a).

All the beneficial bacteria recorded viable potential to enhance K uptake in maize plant most expecially in sterile soil. This could be probably due to the fact that all the beneficial bacteria had nitrogen and phosphorus uptake above the mean value, which eventually regulate and enhance K uptake in maize plant (Hussaini *et al*., 2008) as was also documented by Ayodele and Omotosho, (2008) that, K uptake increases with N and P fertilization. However, mechanisms of N, P and K uptake in maize plant is not part of this study, but agreed that, the predominance of the mechanisms depends on the degree of soil weathering (Vega, 2007), soil pH, root exudates, variation in soil types, plant genotypes (Zaidi *et al*., 2009).

Variation were observed in correlation of maize plant growth parameters such as plant height, stem girth, number of leaves and leaf area in both sterilized and unsterilized soil. The variability in this correlation could be as a result of plant growth promoting rhizobacteria strains, plant genotype, environmental conditions (Bent *et al*., 2001), fertility status of the soil (Mehrotra and Lehri, 1971) and probabaly change in mechanisms of plant gowth promoting rhizobacteria that are not yet fully understood (Dey *et al*., 2004).

Soil condition influenced growth promotion by bacterial strains (Gholami *et al*., 2009). This could be justified to what was obtainable on maize planted in sterile soil. Obviously, there was low level of microorganisms in the sterile soil, thus, less or no competition is expected from any indigenous soil microflora. This gives the beneficial bacteria an advantage to demonstrate their plant growth promoting characteristics on maize growth. The correlation of bacteria load and maize growth in sterile and unsterile soil significantly ($P < 0.05$) influence the correlation and uptake of nitrogen, phosphorus and potassium which eventually affect the maize plant shoot dry matter. Observation in this study, conform with the hypotheses of Abass and Okon (1993) that IAA and other plant hormones were responsible for increased growth of canola, tomato (Lycopersicon esculentum Mill.) and wheat (*Triticum turgidum* L.) in non – sterile as well as in sterile soil. Similarly, auxins produced by rhizobacteria can influence plants growth, including root development which improve uptake of essential nutrients, thus, increasing plant growth (Vikram *et al*, 2007). This may imply that rhizobacteria had more competitive ability to survive and affect the growth of inoculated plants in the presence of indigenous microflora (Khalid *et al*., 2004). However, N, P, K and dry matter of maize plant followed the same correlation pattern in

an unsterile soil. This revealed that, beneficial bacteria in this study have potential prospects as microbial fertilizer to enhance and support maize growth on the field.

The post – experimental rhizosoil pH varied, coupled with weak correlation with bacterial load. Bacteria grow at different pH, depending on the soil condition, reflecting the general effect of change (H^+) on the rates of enzymatic reaction. The pH affects the changes of bacterial load observed in this study, because, strong acids and bases can be highly damaging to enzymes and other cellular substances (Brock, 1986; Talaro, 2005). The variation of maize growth parameters and nutrient uptake may as well be attributed to the effect of pH on bacterial load and colonization ability.

The shoot dry weigth of maize and N were significantly correlated. The beneficial bacteria inoculated showed positive response and enhance shoot dry matter and N content when compared with the control. This revealed that N content in maize is related to shoot biomass accumulation. This is supported by the report of Lafitte and Edmeades (1994b) that shoot dry matter strongly correlated with shoot N uptake in maize plant. Also, shoot dry weight relationship with P was evident in this study. The implication of this is that, beneficial bacteria play significant role in P solubilization mechanisms and soil pH. With respect to this, it could be inferred that the beneficial bacteria has the potentials to solubilize insoluble phosphates, make it available to maize and thus, for maximum shoot dry weight. However, the leaf chlorosis and leafspot diseases of maize expression in an unsterile soil could probably be related to the negative correlations observed in other maize growth variables and parameters.

Molecular identification of beneficial bacteria reported in this study is very important with reliability, safety and specific identity of isolates prior field application. Biochemical identification of beneficial rhizobacteria, in addition to being laborious and time consuming, is not always diagnostic and may produce results that do not match typical criteria or those for designated type strains. PCR, which allows the specific and sensitive amplification of a preselected DNA region, has been intensively applied to the species identification of numerous organisms (Baracco *et al*., 2001; Kiska *et al*., 2002).

Out of the 19 bacteria isolates, exactly 12 were carefully selected based on their beneficial performance to enhance maize growth and nutrients uptake. These 12 beneficial rhizobacteria

were molecularly characterized using 16SrDNA. From the results of the phylogenetic analysis based on the 16SrDNA gene sequences, beneficial bacteria isolates involve a variety of phylum and genera. Proteobacteria, Bacteriodetes and Firmicutes were the main phyla in this study. The phylum Proteobcteria dominated other phylla, they varied in cultivated soil, presenting a great morphological and metabolic diversity (Smith *et al*., 2001, Pereira *et al*., 2006), and thus, this could be associated with the variation of bacteria observed in cultivated soil of Southwestern Nigeria.

All the isolates sequences of the phylum Proteobacteria did match genera with a confidence level above 80%, with the exception of ABS6 (*Azomonas*) that has a confidence level of 26%. Taxonomic evaluation of polyphasic methods (Vandamm *et al*., 1996) will be needed to determine the exact taxonomic position of ABS6 (*Azomonas*) at the specific level.

Enterobacter (OSR7 and IGGR11) isolated from cultivated soil and rhizosphere of maize showed high chitinolytic and antagonistic activity (Berg *et al*., 2002). Sequence alignment on the phylogenetic tree showed similarity alignment of OSR7 with high identity to *Enterobacter radicincitans*, and IGGR11 as *Enterobacter pyrinus*. The presence of *Enterobacter* from the rhizosphere of maize in this study; agreed with the report of Morales – Garcia *et al*. (2011) who isolated *Enterobacter cloacae* from rhizosphere of maize in Mexico, Shoebitz *et al*. (2009) isolated *Enterobacter* sp. from the rhizosphere of *Lolium perenne*, Mehnaz *et al*. (2001) reported that of rice rhizosphere, Yoon *et al*. (1996) reported for soils near the roots of Leguminous plants, thus showing its natural association to rhizosphere environment. Additionally, from the boot – strap analysis, an interesting observation was found that isolate IBS8 sequences formed a phylogenetic similarity with *Citrobacter* group, suggesting that, isolate IBS8 sequences could be a unique strain most closely related to *Citrobacter braaki* and *Citrobacter fameri*. This suggests that, the occurrence and possibility that plant associated *Citrobacter* (Ansari *et al*., 2000) could display plant growth promoting abilities in Southwestern Nigeria is interesting.

The occurrence of isolate UNS sequences in this study shared a low level of phylogenetic sequence similar to *Stenotrophomonas maltophilia*. Although, phylogenetic relationship with *S. maltophilia* was low, but formed a phylogenetic lineage related to the family of Xanthomonadaceae. Similarly, the sequence identity of *Stenotrophomonas* from Southwestern

Nigeria shared the same biotechnological importance to that of Berg *et al*. (1999) isolated in Germany.

Phyllum Bacteroidetes had 33.33% of the total beneficial bacteria. The presence of Bacteroidetes in this study confirmed the report of Borneman *et al*. (1996); Borneman and Triplet (1997); Dunbar *et al*. (1999) in cultivated soil of United States of America. Thus, the presence of Bacteroidetes in cultivated soil of Southwestern Nigeria showed their ecological impact channeled towards maize growth and nutrients uptake. Interestingly, and consequence to the phylogenetic similarity identity, the 16SrDNA sequences of EPR4, IPR1, AT – IKS and TDS9 matched with *Myroides odoratus* in the phylogenetic relationship. *Myroides odoratus* occurred in virtually all the cultivated soil of the studied ecological zones (Green *et al*., 2001). This justified their potentials to be used as biofertilizer alone or in combination with other beneficial bacteria. However, there is need for further investigation on rhizospheric *Myroides odoratus* in Southwestern Nigeria because they have origin and source so implicated as human pathogens (Bachman *et al*., 1996; Spanik *et al*., 1998), although, Green *et al*. (2001) reported that *M. odoratus* is a rare clinical isolate and is often not considered pathogenic. This study therefore suggests that, it would be good to differentiate strains of *M. odoratus* that are of biotechnological potentials from that of clinical isolates in Southwestern Nigeria.

Phyllum Firmicutes, representing 25% of the total beneficial bacteria in this study; are mostly found in cultivated soil (Tzeneva *et al*., 2004; Pereira *et al*., 2006). Although, they varied from one location to the other but prevail only if there is a great quantity of available nutrients in low competition areas. From the result of sequence alignment in this study, it was observed that sequences belonged to the class Bacilli. From the bootstrap analysis, an interesting observation was found, that EPR7 and ILS13 exhibited high 16SrDNA sequence similarity of 86% and 71% to that of *Bacillus aeolius* and *Bacillus niacini* respectively. Thus, molecular identification of rhizospheric *Bacillus* in this study is remarkable compared to the work of Orole and Adejumo (2011) that limited identification of rhizospheric *Bacillus* of maize plants in Southwestern Nigeria to biochemical identification. So far, in Southwestern Nigeria, this may be the first report on occurrence of *Bacillus aeolius* and *Bacillus niacini* associated with maize plants rhizosphere. This corroborated with the report of Gao *et al*. (2004) who worked on association of different rhizosphere *Bacillus* sp. with 14 maize cultivars. This indicates that, the occurrence of *B. aeolius*

and *B. niacini* in this study is possible as maize has broad host range to accommodate different microorganisms including *Bacillus* coupled with the fact that bacteria isolation was from different ecological zones and different maize varieties in Southwestern Nigeria. Additionally, isolate EPR2 shared low level of sequence similarity with *Lysinibacillus boronitolerans*, although, sequences belongs to the same class (Bacilli) with *Bacillus* but the unique presence of *Lysinibacillus* justified its potentials as plant growth promoting rhizobacteria. Further research is needed to discover other possible potentials of rhizospheric *Lysinibacillus* isolates.

Fresh water swampy forest had the highest beneficial bacteria, compared to Derived savannah earlier reported based on the plant growth promoting characteristics of the isolates, Infact, Lowland rainforest had the same distribution percentage of (25%) with Derived savannah, while Guinea savannah as well shared the same distribution percentage (8.33%) with Mangroove forest. The less number and variation observed in beneficial bacteria across and within the ecological zones could be attributed to a large number of physico-chemical changes taking place in the soil which occur due to agriculture. Soils of Southwestern Nigeria suffer physical degradation, such as erosion and chemical degradation, which causes nutrient loss. These degradations can sometimes be caused by tillage, careless use of pesticides and fertilizers, and sewage slime, which eventually causes organic matter and biodiversity loss (Busse *et al*., 2001; Ibekwe *et al*., 2001; Girvan *et al*., 2003). Moreso, the knowledge of the genetic structure of a bacterial population in the rhizosphere can help in relating its changes to environmental variations over time (Smith *et al*., 1995; Wise *et al*., 1995), thus, led to ecotype selection.

Combined effects of beneficial bacteria in comparison to NPK fertilizer had significant effect on maize plant height, stem girth, number of leaves and leaf area far beyond that of maize plant treated with NPK fertilizer and that of untreated maize plant (maize alone). This suggest that the combined bacteria exhibited their plant growth promoting activity, which may result from different mechanisms such as the production of plant – stimulating growth substances (phytohormones) or the suppression of minor plant pathogens by various mechannisms (Glick, 1995; Van Veen, 1997; Dobbelaere *et al*., 2003). This response of combined bacteria was accompanied by significant increase in shoot dry weigth and other parameters. This is in line with those obtained by (Mohamed and Gomaa, 2005) who stated that biofertilizers treatments increased vegetative growth parameters compared to controls. Similarly, it could as well be

attributed to synergistics effects (Iruthayathas, 1983) of co – inoculation of bacteria. Additionally, observation showed that NPK treated maize plant partly enhances maize growth than untreated maize plant, this agreed with the report of Adediran and Banjoko (2003). Despite the significant effect of NPK fertilizer on maize growth, the environmental consequences and health implications still remains a global subject of debate to sustainable maize growth. The extensive use of chemicals as fertilizers to improve plant health and productivity and for the control of pathogens has disturbed the ecological balance of soil and has lead to the depletion of nutrients (Sharma *et al*., 2007). Interestingly, the leaf area of combined bacteria treated maize plant and untreated maize plant were significantly enhanced (Marambe *et al*., 1994) compared to maize plant treated with NPK fertilizer. This suggests that, good soil and favourable environmental conditions can enhance leaf area of well planted maize.

The soil pH of maize plant treated with combined bacteria and that of untreated maize plant was slightly acidic to neutral, known to be ideal for maize growth in Southwestern Nigeria (Ayodele and Omotosho, 2008). The pH supported and balanced the bacteria population (Higa and Wididana, 1989) in maize rhizosphere, thus, enhance nutrient uptake (Sangakkara *et al*., 1993), maize growth (Oyekanmi *et al*., 2008) and dry matter (Gharib *et al*., 2008; Moawad *et al*., 2005) as well as reduction of leafspot diseases of maize and maize leaf chlorosis. This corroborated with the report of Anderson (1991) that, soil that supplies adequate nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S) and micronutrients with favourable soil pH will produce plant vigour and good yield if other conditions of growth such as biological and physical properties of soil are favourable. Also, the rhizosoil pH of NPK fertilizer treated maize plant was acidic. This suggests that, NPK fertilizer may have influenced the pH (Kashem and Singh, 1998; Chamon *et al*., 2009), thus; limit the effect of combined bacteria towards nutrient uptake (Vega, 2007) and as bioprotectant (Scher and Baker, 1982).

Despite the high amount of N, P, K released to maize plant by the combined bacteria, the unused N, P, K remaining in post – experimental rhizosoil was relatively high. This indicates an understanding that rhizosphere bacteria participate in the geochemical cycling of nutrients and determine their availability for plants and soil microbial community (Vega, 2007) coupled with physico – chemical conditions that predominate in the rhizosphere, may as well be useful to

understand the roles that microorganisms played, particularly bacteria on soil nutrient availability.

CONCLUSION AND RECOMMENDATION

The use of chemical fertilizer to improve maize growth in Nigeria has become a challenge due to its environmental and health implications. In view of this, this study encouraged biological based approach, being environmentally friendly and cost effective. Similarly, this study justified the variation in soil physical conditions and low chemical contents in soils of Southwestern Nigeria, thus, calls for urgent attention.

Plant growth promoting characteristics and phyto-beneficial effects of isolates aided selection of beneficial rhizobacteria coupled with the first known report in Southwestern Nigeria that justified utilization of D-L tryptophan (in-vitro) by soil (rhizo) bacteria and the quantity of IAA produced was far higher than that of L-tryptophan.

The nature of variability in plant growth promoting characteristics and phyto-beneficial effects of rhizobacteria isolates on maize growth, notably nutrients uptake are issues for consideration in assessing these rhizobacteria as biofertilizer. Also, natural association of beneficial rhizobacteria isolates with the rhizosphere of maize, showed their potentials for inoculation attempts in the field.

This present study reported that maize plants in Southwestern Nigeria harbour three different phylla taxonomic groups which are Proteobacteria, Bacteriodetes and Firmicutes. The 16S rDNA molecular-based approach revealed that *Myroides odoratus, Enterobacter pyrinus, Enterobacter radicincitans, Bacillus aeolius, Bacillus niacini, Lysinibacillus boronitolerans, Citrobacter fameri, Stenotrophomonas maltophilia* and *Azomonas macrocytogenes* are common inhabitants of maize rhizosphere in Southwestern Nigeria. Moreso, the use of 16S rDNA molecular technique for proper taxonomic placement of the sequenced beneficial rhizobacteria isolates was extremely useful in this study. Closer analysis of rhizobacteria from other food crops in Southwestern Nigeria may reveal novel sequences. *Myroides* sp. and *Lysinibacillus* sp. were reported for the first time in this study as phyto-beneficial rhizobacteria of maize plants in Nigeria.

The phyto-beneficial rhizobacteria are promising for sustainable maize growth in Southwestern Nigeria, hence, could be considered as a suitable substitute for chemical fertilizer in organic agricultural systems.

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