

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



**Sudan University of Science and Technology**

**College of Graduate Studies**



**The Effect of Compost, Rhizobium Inoculation and Urea on  
Peanut Plant Growth**

**(Arachis Hypogea)**

**تأثير إضافة السماد العضوي وبكتيريا الرايزوبيوم والسماد الكيميائي على نمو  
نبات الفول السوداني**

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## الآية

بسم الله الرحمن الرحيم

قال تعالي في محكم تنزيله:

(وَإِنْ كُلُّ لَمَّأٍ جَمِيعٌ لَدَيْنَا مُحْضَرُونَ \* وَآيَةٌ لَهُمُ الْأَرْضُ الْمَيْتَةُ أَحْيَيْنَاهَا  
وَأَخْرَجْنَا مِنْهَا حَبًّا فَمِنْهُ يَأْكُلُونَ \* وَجَعَلْنَا فِيهَا جَنَّاتٍ مِنْ نَخِيلٍ وَأَعْنَابٍ  
وَفَجَّرْنَا فِيهَا مِنَ الْعُيُونِ \* لِيَأْكُلُوا مِنْ ثَمَرِهِ وَمَا عَمِلَتْهُ أَيْدِيهِمْ أَفَلَا يَشْكُرُونَ  
\* سُبْحَانَ الَّذِي خَلَقَ الْأَزْوَاجَ كُلَّهَا مِمَّا تُنْبِتُ الْأَرْضُ وَمِنْ أَنْفُسِهِمْ وَمِمَّا لَا  
يَعْلَمُونَ)

صدق الله العظيم

(يس الآية 32 - 36)

## **Dedication**

*To My Mother*

*To My Father*

*To My Brother*

*To My Sisters*

*To My Friends*

*To all My Colleagues'*

*To All My Professors at school My Tracks*

## Acknowledgments

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## **Abstract**

The Rhizobium isolate ENRI 24 Was highly affected by the high salt concentration( 0.6,0.8,1g/L) and (2 g/L), the Rhizobium colony counts were  $10^4$ cfu/ml and  $10^8$  cfu/ml.

The Rhizobium strain ENRI 24 was also affected by high pH concentration. Concerning the effect of temperature, The optimum growth was found on temperature30C in both of  $10^4$  and  $10^8$  ml.

In the laboratory study that the growth rate of Rhizobium varied according to the concentrations of different salts, pH values as well as different temperature in such study the number of Rhizobium ranges between 0-320 CFU/ml.

The study was conducted in both laboratory and green house facility of the College of Agricultural Studies (CAS). Sudan University of Science and technology (SUST) in Shambat to study the effect of organic fertilizer, Rhizobium inoculation, and chemical fertilizer on peanut plants growth, and to study also the effect of salinity, pH, and temperature on the bacteria of Rhizobium growth in laboratory.

The results of the study showed that compost is the best compared with the control. It gave positive effect on peanut plant shoot and root dry weight. However ,The treatments 0.8g/5kg soil and 3g/5kg soil gave the highest values of shoot and roots lengths of the plant respectively.

In addition, high values were found in the number of the main and lateral nodules at the concentration of Rhizobium  $10^4$ cfu/ml and  $10^8$  cfu/ml respectively. NPK was significantly high compared to the control in treatments of Rhizobium at  $10^4$ cfu/ml and  $10^8$ cfu/ml.

## ملخص البحث

أجريت كل من المعمل والبيت المحمي بجامعة السودان للعلوم والتكنولوجيا، كلية الدراسات الزراعية لدراسة تأثير الملوحة و الأسم الهيدروجيني والحرارة على نمو بكتيريا الرايزوبيوم . تأثير العضوي وبكتيريا الرايزوبيوم الكيميائي أن بكتيريا الرايزوبيوم ابدت تأثير .

30C فاع الأسم الهيدروجيني، وفيما يتعلق بدرجة مئوية كيزين (  $10^8$   $10^4$  ) أظهرت نسبة نمو الرايزوبيوم تباينت عند درجات تراكيز الهيدروجيني والدرجات الم

الرايزوبيوم بين 0-320

السماذ العضوي هو بالشاهد اذ اثر ايجابي علي وسيقان وجذور حيث كانت اعلي قيمة بالنسبة لطول النبات وطول الجذر عند 0.8 3 5 قيم على طول

وسيقان وجذور النبات علي التوالي. لهذا القيم لوحظت على عدد العقد الرئيسية والفرعية عند تراكيز ( $10^8$   $10^4$ ) . تركيز نيتروجين و بوتاسيوم أظهرت النتائج فروقات ذات معنوية عالية مقارنة بالشاهد عند تراكيز ( $10^8$   $10^4$ ) ن عينة بكتيريا الرايزوبيو

. 24

# CHAPTER ONE

## INTRODUCTION

The peanut, also known as the groundnut and the goober and taxonomically classified as *Arachis hypogaea*, is a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. It is classified as both a grain legume and, because of its high oil content, an oil crop. World annual production of shelled peanuts was 42 million tonnes in 2014. Atypically among crop plants, peanut pods develop underground rather than aboveground. It is this characteristic that the botanist Linnaeus used to assign the specific name *hypogaea*, which means "under the earth."

As a legume, the peanut belongs to the botanical family Fabaceae; this is also known as Leguminosae, and commonly known as the bean, or pea, family. Like most other legumes, peanuts harbor symbiotic nitrogen-fixing bacteria in root nodules. This capacity to fix nitrogen means peanuts require less nitrogen-containing fertilizer and improve soil fertility, making them valuable in crop rotations.

Peanuts are similar in taste and nutritional profile to tree nuts such as walnuts and almonds, and are often served in similar ways in Western cuisines. The botanical definition of a "nut" is a fruit whose ovary wall becomes very hard at maturity. Using this criterion, the peanut is not a true nut, but rather a legume. However, for culinary purposes and in common English language usage, peanuts are usually referred to as nuts.

Peanuts grow best in light, sandy loam soil with a pH of 5.9–7. Their capacity to fix nitrogen means that, providing they nodulate properly, peanuts benefit little or not at all from nitrogen-containing fertilizer, and they improve soil fertility. Therefore, they are valuable in crop rotations. Also, the yield of the

peanut crop itself is increased in rotations, through reduced diseases, pests and weeds.

( <https://en.wikipedia.org/wiki/Peanut> 2017 Access on 18 February, at 04:35.)

Until the second half of 2002, Sudan's economy boomed on the back of increases in oil production, high oil prices, and large inflows of foreign direct investment.

Agricultural production remains important, because it employs 80% of the work force and contributes a third of GDP. The Darfur conflict, the aftermath of two decades of civil war in the south, the lack of basic infrastructure in large areas, and a reliance by much of the population on subsistence agriculture ensure much of the population will remain at or below the poverty line for years despite rapid rises in average per capita income. Sesame seeds and peanuts are cultivated for domestic consumption and increasingly for export. However, Sudan remains a net importer of food. Problems of investment finance, production and transportation remain the greatest constraints to a more dynamic agricultural economy.

Sudan has 84 million hectares of arable land and less than 20% is cultivated. Major agricultural projects such as the Gezera Scheme in Gezira state are underway in order to make Sudan food self-sufficient. Sudan is one of the world's potential breadbaskets and Sudan is nicknamed as the Arab world food basket as it accounts for 45% of arable land in the Arab world ([https://en.wikipedia.org/wiki/Economy of Sudan](https://en.wikipedia.org/wiki/Economy_of_Sudan) May 2013)

A Bio fertilizer is a substance which contains living microorganisms, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical

fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. (<https://en.wikipedia.org/wiki/Biofertilizer> March 2016)

Rhizobium is a genus of Gram-negative soil bacteria that fix nitrogen. Rhizobium species form an endosymbiotic nitrogen-fixing association with roots of legumes and Parasponia.

The bacteria colonize plant cells within root nodules, where they convert atmospheric nitrogen into ammonia and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. The plant, in turn, provides the bacteria with organic compounds made by photosynthesis. This mutually beneficial relationship is true of all of the rhizobia, of which the Rhizobium genus is a typical example.

(<https://en.wikipedia.org/wiki/Rhizobium> 2017, access 15 January)

**Compost** is an organic matter that has been decomposed and recycled as a fertilizer and soil amendment. Compost is a key ingredient in organic farming.

At the simplest level, the process of composting requires making a heap of wetted organic matter known as green waste (leaves, food waste) and waiting for the materials to break down into humus after a period of weeks or months. Modern, methodical composting is a multi-step, closely monitored process with measured inputs of water, air, and carbon- and nitrogen-rich materials. The decomposition process is aided by shredding the plant matter, adding water and ensuring proper aeration by regularly turning the mixture. Compost is rich in nutrients. It is used in gardens, landscaping, horticulture, and agriculture. The compost itself is beneficial for the land in many ways, including as a soil conditioner, a fertilizer, addition of vital humus or humic acids, and as a natural pesticide for soil.

(<https://en.wikipedia.org/wiki/Compost> January 2017).

Various chemicals are used to enhance the efficiency of nitrogen-based fertilizers. In this way farmers can limit the polluting effects of nitrogen runoff. Nitrification inhibitors (also known as nitrogen stabilizers) suppress the conversion of ammonia into nitrate, an anion that is more prone to leaching. Urease inhibitors are used to slow the hydrolytic conversion of urea into ammonia, which is prone to evaporation as well as nitrification.

The conversion of urea to ammonia catalyzed by enzymes called ureases.

<https://en.wikipedia.org/wiki/Fertilizer> on 24 February 2017, at 05:21.

### **Objectives of the study:**

The main objectives of this study are:

1. To test the effect of salinity ,temperature, and pH on Rhizobium.
2. To investigate the effect of adding compost on the plant growth.
3. To test the effect of adding Rhizobium inoculation on the plant growth.
4. To compare the effect of chemical fertilizer with organic and Rhizobium inoculation on plant growth.

# CHAPTER TWO

## LITERATURE REVIEW

### **Background:**

#### **2-1 Composition of Manure**

There is a great deal of variation in the composition of manure. The variation depends on such diverse factors as the kind and age of livestock, The phosphorus content of manure is relatively low compared with plant needs. Furthermore, about 30% of the phosphorus (and up to 80% of the nitrogen and roughly half of some other nutrients) in manure is not immediately available for plant growth (Egball *et al.* 2002). Mixtures of soil and decomposing organic matter is called compost and often used by gardeners as a fertilizer. Usually a pile is made by alternating layers of soil or sod with organic materials such as manure, garbage, grass clippings, and so on.

Manure is primarily a nitrogen-potassium fertilizer. It releases its nutrient fastest when the soil provides warm and moist conditions which are favorable for microbial decomposition. Manure applications are therefore most effective on a warm-weather crop needing nitrogen and potassium.

Manure are normally applied at rates of a few tons per acre. The largest crop yield increases at relatively low rates of the applied manure. But the largest profit per acre resulting from higher rates of manure applications. (FrederickR, *et al.* 2005)

Large animal-feeding operations produce so much manure. However, they generally do not own enough land to use their manure efficiently (Ribaud, *et al.* 2003). Many of them apply it to their land at high rates which allow it to accumulate in or near the feedlots.

Gamar (2004) showed that adding of chicken manure fertilizer to teff gave positive results similar to those of urea, so it can be used as alter native to chemical fertilizers. Teff forage yield was significantly improved by urea and chicken manure fertilization.

## **2.2 Composting:**

The breakdown of organic matter during composting process depends on several factors working in concert, these included moisture, microbial, populations, oxygen and carbon (C, nitrogen (N) ratio.

The nitrogen of matures Compost is mineralized into ammonium and then converted to nitrate which are the preferred forms for plant up take (Nathan, 2004).

## **2.3 Composting process:**

Composting may be divided into the following two categories based on the nature of the decomposition process.

### **2-3-1-Anaerobic composting:**

Anaerobic composting-decomposition occurs where oxygen ( $O_2$ ) is absent or present in limited supply, under these condition anaerobic microorganisms dominate and develop intermediate compounds including methane, organic acid, hydrogen sulphide and other substances.

In the absence of oxygen these compounds accumulate and are not metabolized any further. However anaerobic composting is a low temperature process.

### **2-3-2-Aerobic composting:**

In this process aerobic microorganisms break down the oxygen takes place in the presence of organic matter and produce carbon dioxide, ammonia, water, heat and humus (The relatively stable organic end Product).



## **2-4 Phases of aerobic compost:**

**It is divided into the following four stages:**

### **2-4-1-Thermophilic:**

During the first day or two days of aerobic composting, labile organic matter carbon is initially degraded by bacterial enzymes present in mesophilic microflora flourishing at temperatures between 50-70 °C. This stage is called the thermophilic stage or active composting.

### **2-4- 2. Mesophilic:**

The aerobic composting processes start with the formation of the pile. The temperature rises rapidly to 70 to 80 °C within the first few days. During this time mesophilic organisms with optimum growth temperature range of 45-50 °C dominate the medium and multiply rapidly consuming the readily available sugar and amino acids and release heat that raises the temperature to a point where their own activities become suppressed (Hamad, 2009).

### **2-4-3-Cooling down:**

When the temperature falls, mesophilic organisms re invade the substrates.

### **2-4 - 4-Maturity stage:**

The degree of maturity required in compost depends on where the product will be put, for mulching on the surface of the ground between rows of crops or around established trees and shrubs, immature compost is perfectly acceptable. (Vltcho., *et al.*, 2004).- suggested that at maturity the compost had a moisture content 66%, 7.7%,

They also suggested that compost could be considered mature if it meets two of the following three requirements:

- C:N ratio of less than 25:1

- Oxygen up take rate of less than or equal to 50 mg.
- Germination of cress (*Lepidium sativum*) seed and radish (*Raphanus sativus*).

Seeds in the compost must be greater than 90% of the germination rate of the control and if the seeds are grown in a mixture of compost and soil the percentage must not differ by more than 50% of the control.

## **2-5 Physical and chemical properties of compost:**

### **2-5-1-Aeration,moisture,and temperature:**

The continuity of metabolic process depends on sufficient aeration. Average O<sub>2</sub> concentration of the compost mixture is 15 to 20%, while CO<sub>2</sub> is 0.5 to 5%. When O<sub>2</sub> content falls below these levels, anaerobic microbial populations surpass aerobic species, and as a result malodorous fatty acids and methane levels may increase Druilhe, *et al.*, (2002).This oxygen consumption is a function of microbial activity and its level is also related to substrate temperature. good aeration and temperature regulation can be achieved by adequate turning.

Too low moisture content ,The water needed for microorganism Metabolism.

Too high moisture content increase, means space will be filled with leading to anaerobic condition.

### **2-5-2- Carbon and Nitrogen dynamics:**

Both cellulose and hemicelluloses fractions remained constant during the first week of thermophilic composting until soluble C was metabolized by microbes.

Brady and Weil (2002) reported that the nitrogen dynamics during composting are largely related to microbial activity.

Most N in composted substrate is in the organic form, mostly as proteins and simple peptides. During the initial rise in temperature heterotrophic microbes' attack the amine groups present in organic matter as humus or proteins, forming amino compounds. Following hydrolyzation, N is either released as ammonium ( $\text{NH}_4^+$ ) or is immobilized by other microorganisms and returned to its organic form.

When substrate's temperature exceeds 40°C or if pH rises above 7.5, N generally volatilizes as ammonia ( $\text{NH}_3$ ). Nitrate ( $\text{NO}_3^-$ ) concentrations in the compost are low during this thermophilic due to  $\text{O}_2$  consumption by the nitrifiers (Nathan, 2004).

### **2-5-3- Heap pH:**

The amount of ammonia lost from a compost heap can be reduced by adding few soil, about 1% of the weight of the heap, and mixing it well with other ingredients. In some heap systems where bamboo poles are used to make air channels, the heap is covered humid white, this helps to reduce ammonia loss. Hamad (2009), reported that the pH of the compost decreased slowly, then increased after the 10th week due to accumulation of ammonia. Compost opening time was best after three months when its volume is very much reduced, due to full decomposition.

### **2-5-4- Compost Salinity:**

Soluble salt levels in compost can vary considerably, depending on feed stock and processing. Compost may, therefore, contribute to or dilute the accumulative soluble salt content in the amended soil.

Knowledge of soil salinity, compost salinity, and plant tolerance to salinity is necessary for the successful establishment of plants for most turf and landscape plantings the final salinity (Ece) of the amended soil should be less than 4.0 ds/m.

Soluble nutrients, particularly potassium, calcium and nitrogen, typically account for most of the salinity in compost products. Sodium is an undesirable soluble salt constituent. This element should ideally account for less than 25% of the total soluble salts in compost according to (William ,*et al*, 1990)

### **2-5-5-Nutrient content:**

A carbon-to-nitrogen ratio (C/N ratio or C:N ratio) is a ratio of the mass of carbon to the mass of nitrogen in a substance. It can, amongst other things, be used in analysing sediments and compost. A useful application for C/N ratios is as a proxy for paleoclimate research, having different uses whether the sediment cores are terrestrial-based or marine-based. Carbon-to-nitrogen ratios are an indicator for nitrogen limitation of plants and other organisms and can identify whether molecules found in the sediment under study come from land-based or algal plants.[1] Further, they can distinguish between different land-based plants, depending on the type of photosynthesis they undergo. Therefore, the C/N ratio serves as a tool for understanding the sources of sedimentary organic matter, which can lead to information about the ecology, climate, and ocean circulation at different times in Earth's history.[2]

When composting, microbial activity utilizes a C/N ratio of 30-35:1 and a higher ratio will result in slower composting rates.[10] However, this assumes that carbon is completely consumed, which is often not the case. Thus, for practical agricultural purposes, a compost should have an initial C/N ratio of 20-30:1.

However, for more practical applications, desired C/N ratios can be achieved by blending common used substrates of known C/N content, which are readily available and easy to use. [https://en.wikipedia.org/wiki/Carbon-to-nitrogen\\_ratio](https://en.wikipedia.org/wiki/Carbon-to-nitrogen_ratio) on 24 November 2016 access, at 17:38.

Hamad (2009) showed that nitrogen, phosphorus, calcium, magnesium, and sodium content increased significantly in the plots treated with composted farm yard manure the nitrogen increase in the composted material was noticed between the 3<sup>rd</sup> and 5<sup>th</sup> week and decreased significantly after 12<sup>th</sup> week.

Similarly, Warman, *et al.*,(2002) observed 42 to 85 increases in total N in three vermicomposted wastes after 45 and 68 days, respectively. After 90 days, however, total N concentration had returned to levels only slightly above Initial concentration.

## **2.6 Phosphorus:**

Hamad (2009) reported that phosphorus content increased significantly up to 8<sup>th</sup> week in the farm yard manure (FyM) treated with chicken manure(CHM) compared to the control, then the P content decreased significantly up to 12<sup>th</sup> week. The significant increase in calcium content was between 6<sup>th</sup> and 12<sup>th</sup> weeks. Magnesium content increased up to 9<sup>th</sup> week and significantly reduced after the 10<sup>th</sup> week.

## **2.7 Potassium and other micro nutrients:**

Hamad (2009) showed that potassium content in samples of composted with FYM decreased significantly with time compared to the control.

## **2.8 Biotic factor:**

Lignin and cellulose:

High levels of cellulose content has been detected through the active phase of composting(Cunba Queda *et al.*,2004).

Goyal *et al.*, (2005) showed that sugarcane trash has more recalcitrant carbon due to presence of large amount of lignin and have low cellulose contents as compared to water hyacinth.

## **2-9 Effect on the physical properties:**

Pellegrini., (2005) showed that the influence of organic amendments on soil physical properties is dependent on soil properties.

## **2.10 Bulk Density and Infiltration rate:**

Barzege *et al.*, (2002) stated that application of organic materials significantly decreased soil bulk density.

## **2.11 Effect on chemical soil properties:**

Soil PH and Ec were increased rate of application decompost. addition of compost to soil may modify the PH of the final mix. (Zheljazkov *et al.*, 2003).

The addition of compost to soil may modify the pH of the final mixture, also Compost addition increases soil PH and electrical conductivity (Vltcho., *et al.*, 2004).

## **2.12 Effect on biological soil properties:**

Henry (2005) stated that compost-C is the energy source for soil micro-organisms and the population of fungi, actinomycetes, and bacteria.

## **2.13 Effect of incorporation of compost on crop yield:**

Barzegar *et al.*, (2002) stated that application of organic materials had significantly increased wheat yield, They also found that composted baggasse increased wheat grain yield by 14% Over the control.

Canali *et al.*,(2004) reported that effect of long term addition of compost had increased soil available nutritive elements to crops, and hence increased yield.

The potential uses of biofertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance productivity. On the other hand, the value of organic materials as a

source of plant nutrients is greatly enhanced by composting. Composted materials are also more stable and pleasant to handle. (Elhassan, *et al.* 2010).

#### **2-14 Ground nut plant:**

Ground nut (*Arachis hypogaea*) belongs to the family Leguminosae. It originated in south America, and has been cultivated since ancient times by native Americans. Plants grow to about 75 cm (about 30 inches) high and can spread 1.2m (4ft). Some types develop a bunched/erect growth. Others, called runners, spread over the ground. In Pakistan, it is cultivated mainly in rain fed (barari) areas of Punjab and also irrigated areas of the Khyber Pakhtunkhwa and Sindh. Ground nuts require a warm growing season of 120 to 140 days, moderate rainfall and a pH of 6.0 to 6.5 is more suitable for its cultivation. The seeds contain 40 to 50 percent oil and 20 to 30 percent protein, and they can be an excellent source of B vitamins (Pardee, 2002).

#### **2.15 Nitrogen Fixation in ground nut:**

Cultivation of groundnut in Western Sudan is still lacking nitrogen fertilizers. Hence three imported strains were compared to local strains. The results indicate that, the imported *Rhizobium* strain has no benefits for groundnut production in Western Sudan. Hence, the future research on nitrogen fixation by groundnut in this area should be directed to selection and identification of the most effective rhizobia strains from the adapted local population (Ali, 2003).

As nitrogen is concerned, crop rotations including a legume have shown, in many cases, very significant benefits for the yields of accompanying or subsequent non-legume grain crops (Bunch, 1999).

The  $\delta^{15}\text{N}$  natural abundance technique can be applied to quantify biological nitrogen fixation (BNF) to any legume, and has even been applied to  $\text{N}_2$ -fixing non-legumes (Boddey *et al.*, 2001).

However, inoculation with a compatible strain of rhizobia was found to enhance nodulation, dry weight of nodules, nitrogen fixation and yield of alfalfa (*Medicago sativa*), Fenugreek (*Trigonella foenugraecum*), cluster bean (*Cyamopsistetragonoloba*), field pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*) grown in dry land. It was concluded that, the productivity of leguminous crops in dry land could be improved by *Rhizobium* inoculation (Abdelgani *et al.*, 2003).

### **2-16 The Rhizobium culture:**

The *Rhizobium* culture strains are antigenically very selective and require particular host. The surface antigen on the *Rhizobium* cells recognizes the binding sites (specific root exudates) on the roots of the leguminous plants. This characteristic makes them host-specific. Specific *Rhizobium* cell can penetrate the roots of the specific leguminous plants only and form nodules. They multiply within the nodule using the carbon source from the plant and in turn fix part of the atmospheric nitrogen to the plant.

### **2-17 Rhizobium characteristics:**

Negative, colloidal, no spores, motionless and the color is blue.

The first known species of rhizobia, *Rhizobium leguminosarum*, was identified in 1889, and all further species were initially placed in the *Rhizobium* genus. However, more advanced methods of analysis have revised this classification, and now there are many in other genera. Most research has been done on crop and forage legumes such as clover, alfalfa, beans, peas, and soy; more research is being done on North American legumes.

The word rhizobia comes from the Ancient Greek  $\rho\acute{\iota}\zeta\alpha$ , *rhíza*, meaning "root" and  $\beta\acute{\iota}\omicron\varsigma$ , *bios*, meaning "life". The word *rhizobium* is still sometimes used as the singular form of rhizobia.



Rhizobia are a paraphyletic group that fall into two classes of the proteobacteria—the alpha- and beta-proteobacteria. As shown below, most belong to the order Rhizobiales, but several rhizobia occur in distinct bacterial orders of the proteobacteria.

## **2-18 The Effect of temperature, salinity, and pH on growth of Rhizobium:**

Legumes are economically important crops and serve as sources of nutritious food, feed and raw-materials for humans, animals and industries respectively. Additionally, legumes have a symbiotic association with nitrogen-fixing rhizobia present in the root nodules, thus plants do not require external nitrogen sources. <https://doi.org/10.3389/fpls.2016.0095729> June 2016

the widely accepted benefits of legumes in cropping systems are needed now more than ever (Arnoldi *et al.*, 2014; Araujo *et al.*, 2015). Legumes/pulses are very important food and feed crops, known for their health benefits (Arnoldi *et al.*, 2014).

Most rhizobia have an optimum growth temperature at 28-31°C and many of them are unable to grow at 38°C (Graham, 1992).

Temperature can influence not only the survival of free ,but also the exchange of molecular signals between the symbiotic partners(sadowsky, 2005).

High temperature can induce an inhibiting effect on bacterial adherence to root hairs, on bacteriod differentiation, on nodule structure and on legume root nodules functioning (Zahran, 1999).

Symbiotic nitrogen fixation may be adversely affected by saline environments. This dissertation describes experiments that assess the salt sensitivity of Rhizobium as free living organisms In addition, a split-root plant growth system is described which can be used to separate the effects of

salinity stress on host yield potential from the effects of salinity on nodule processes.

Nitrogen fertilization improved dry matter production but depressed nodulation. Phosphate mitigated the depressive effect of nitrogen on nodulation and further enhanced its stimulatory effect on dry matter production, Investigation on nodulation of guar and five other species of legumes (*Cajanus cajan*, *Vigna unguiculata*, *Crotalaria saltiana* and *cassiaoccidentalis*) in Sudan in spite of these legumes were naturally nodulated, inoculation by introduced or locally-isolated bacterial strains improved nodulation and dry matter production (Mahdi and Mustafa, 2005).

The response to saline stress varies among free rhizobia for which the growth is inhibited at 100 mM NaCl, and symbiotic rhizobia, such as *Sinorhizobium meliloti* found to be tolerant to NaCl concentration ranging from 300 to 700 mM (Zahran 2001)

The optimum pH for rhizobial growth is considered to be between 6.0 and 7.0 (Hungria, and Vargas 2000).

The rhizobial strains vary widely in their acidity tolerance. Rhizobial tropici is considered as highly acid tolerant strains (Graham, *et al.* 1994).

Some rhizobial strains can withstand and survive even at a very low pH about 3.5.

Alkalinity is less harmful to the survival of rhizobia. Joedan 1984 showed that the majority of these bacteria can tolerate up to pH 9.

The medium pH of plant tissue cultures has been shown to be very important to many aspects of explant development and growth.

After placing explants on the medium, medium pH fluctuations have also been reported for various species.

According to Skirvin *et al.* (1986) and Thorpe *et al.* (2008), explant nutrient absorption in vitro is a function of ion exchange where deposition of free hydrogen ions (H<sup>+</sup>) and hydroxyl ion (OH<sup>-</sup>) in the medium may contribute to acidic or alkaline medium pH. Organic, inorganic salts, amino acids, vitamins, sucrose, gelling agents, and plant growth regulators are the common components added to tissue culture medium. Williams *et al.* (1990) reported that adding agar significantly elevated medium pH prior to autoclaving when pre-adjusted pH ranged from 3.5 to 5.5 in medium. Additions of synthetic or natural organic acids generally increase medium buffering ability (Thorpe *et al.*, 2008). Organic compounds such as (N-morpholino) ethanesulfonic acid (MES) are known to help maintain suitable medium pH range for explant development (de Klerk *et al.*, 2008; Parfitt *et al.*, 1988; Yuan *et al.*, 2012).

# **CHAPTER THREE**

## **MATERIALS AND METHODS**

### **Compost experiment:**

#### **3.1 Location of experiment:**

The experiment was conducted in demonstration field, College of Agricultural studies, Sudan University of Science and Technology. Shambat

#### **3.2 Management of composting process:**

Alfalfa(14kg), grass (16.5kg) and cow manure (27kg) was added by plate weight intervals. The amount of water added (10.5 Liter) every (week) depends on moisture content. The compost aerated every week by turning composting materials and taking samples.

#### **3.3 Compost sampling:**

Samples were taken every week from three depths top, middle and the bottom.

#### **3.4 The analysis of compost:**

The analysis for compost was:

- Nitrogen analysis by using kjeldahl method.
- Phosphour analysis by using oslen method
- Organic carbon by using walkely and black .

#### **3.5 Compost duration:**

The composting process was stayed about four months inside the pit, with addition of water as required.

## **In vitro experiment:**

### **3.6 Rhizobium strain:**

The *Rhizobium* strain (ENRI 24) was obtained from the Environment and Natural Resource Research Institute, The National Center for Research, Sudan. Erlenmeyer flasks containing 200 mls of sterile yeast extract mannitol agar broth (YEMA), were inoculated with the *Rhizobium* strain under aseptic conditions. The flasks were placed on an orbital shaker at 200 rpm at ambient temperature for three days. (Howieson *et al.*, 2004). Serial dilutions were made to count the Rhizobium cells/ml. approximately ( $10^4$  - $10^8$  CFUs).

### **3-7 Plate count method:**

Done Sterilized the water in tubes with petry dishes, media in atuoclave and taking 1ml of the vaccine and put in first tube taked 1 ml at first tube and put in to the second tube and so on untl it reached the eight tube(series dilution).

After that Done taking 0.5 ml at the fouth tube put in petry dish  $10^4$  , taking 0.5 ml at eight tube but in petry dish  $10^8$  .

Then count the Rhizobium after growth.

### **3-8 Constituents of YEMA:**

- 1- Magensium sulphate 0.1 g
- 2- Potassium dihydrogen 0.1 g
- 3- Yest Extract 0.2 g
- 4- Dipotassium hydrogen phosphate
- 5- Agar 20 g
- 6- Mantol 10 g
- 7- Scharalu

### **3.8 Strain preservation:**

*Rhizobium* strains were maintained on Yeast Extract Manitol agar (YEAM) slants in the fridge.

### **3.9 Effect of temperature on Rhizobium growth:**

*Rhizobium* was incubated at different degrees of temperature (20-30-40 C )for three days and three replicates for each treatment were prepared.

### **3-10 Effect of pH on Rhizobium growth:**

The ability of rhizobium to grow at different pH levels was studied by using the following pH values (6-7-8-9) with three replicates for each treatment). The pH was determined using pH meter (model 3510) .

### **3.11 Effect of salinity on Rhizobium growth:**

The ability of rhizobia to grow on different concentrations of NaCl was studied by using YEMA medium in which the following NaCl concentrations (0.2-0.4-0.6-0.8-1-2 g/L) with three replicates for each treatment) and a control (with no NaCl other than the 0.1 g/1000 ml required by the microbe for healthy growth ) Three days later, the results were recorded .

### **3.12 Pot experiment:**

The study was conducted in green house in June 2015, at Sudan University of Science and Technology, College of Agricultural Studies (CAS).

Seeds of groundnut; (Valencia variety) were obtained from the Department of Crop Protection (CAS). Then they were surface sterilized by H<sub>2</sub>O<sub>2</sub> (3%) for 15 minutes and washed three times by sterile water. The sterilized seeds were then transferred to petri dishes and incubated at 30°C for four days in an incubator model (LIB030M). Shambat top soil was sieved using a 2-mm mesh sieve and then sterilized at 180°C, for 2 hours using the oven. This was to eliminate microorganisms. The physical and chemical characteristics of the

soil were determined including electrical conductivity, soil reaction (pH), soluble cations, soluble anions (Richard 1954), total nitrogen (Ryan, *et al.*, 2001), organic carbon, phosphorus by (Olsen method 1954), mechanical analysis were determined by using hydrometer method (Days, 1956), and sodium adsorption ratio by flame photometer. Black plastic bags (20cm diameters, five kilogram capacity) were filled with 4 kg or 5kg of a sterilized soil. Drainage holes were made in the bottom of the bags using a sterile needle. Eight seeds were aseptically added per bags. The bags were irrigated immediately with a sterile tap water. For all treatments, seeds were placed at 5-cm depth from the soil surface. For the *Rhizobium* treatments, one ml of the *Rhizobium* inoculum culture was added to the sown seeds, as previously described by (Azcon *et al.* (1991). For the composting treatment 0.8, 1.6, 3g/5Kg soil For the fertilizers treatments urea was used at the recommended dose (43kg/ha.) (Farah and Eastin, 1988). Plants were thinned to five plant per pot following seedlings emergence. Plants color were rated after one, two and three month and plant height measures were done after three month. Soils were maintained at moisture holding capacity. Pots were randomized in the shade and repositioned once a week.

The treatments applied for groundnut were as follows: (Urea) with phosphorus mineral fertilizer, nitrogen mineral fertilization (Urea), phosphorus mineral fertilization and Control no inoculation no fertilization.

### 3-13 Treatments used:

Compost 0.8	Compost 0.8g/5kg soil(0.008Kg)
Compost 1.6	Compost 1.6g/5kg soil(0.0016 Kg)
Compost 3	Compost 3g/5kg soil(0.003Kg)
Nitrogen N <sub>1</sub>	Urea 0.0001g/5kg soil(23Kg N/fedan)
Nitrogen N <sub>2</sub>	Urea 0.0002g/5kg soil(46Kg Kg/ fedan)
Control	Control W/O inoculation W/O fertilization
Rhizobium 10 <sup>4</sup>	Dilution of alien Rhizobium (Enrri24) bacteria(1 ml/5kg soil)
Rhizobium 10 <sup>8</sup>	Dilution of alien Rhizobium (Enrri24) bacteria(1 ml/5kg soil)

### **3.14 Data collection:**

When the plants reached physiological maturity, the following growth characters were measured.

### **3-15 Growth characters:**

- 1- The plant height (**cm**):
- 2- Root dry weight
- 3- Shoot dry weight
- 4- Basic Nodules number
- 5- Lateral Nodules number
- 6- Basic Nodules weight
- 7- Lateral Nodules weight
- 8- NPK (%)

### **3.16 Tissue analysis:**

The weight and count of nodules were made at the flowering stage. The plants dried in the hot air oven with the temperature at  $72^{\circ}\text{C}$  for 48hour. The top dry and root weights were determined for Ash content of the different sample at 24hour following ,The standard method as described by (Ryan, *et.al*, 1996).

Plant samples were extracted using 10ml of hydrochloric acid(5N) and put in a hot sand bath until bubbles appear complements for 10 minutes.

The extract was filtered and made to 50ml volume.

Then take 10ml and add 10ml of ammonium molybdate+nitric acid and supplements to 100ml volume.

Add 1ml of the sulfuric acid, also add a point of stannous chloride(show color solution).

**The analysis of the plant after burning determined the NPK as follows:**

Nitrogen content was determined using kjeldhal method (Ryan *et.al*,1996)



Phosphorus was determined using aspectrophotometer (Ryan *et.al*,1996).

Potassium was determined using aflame photometer(Ryan *et.al*,1996).

### **3.17 Statistical analysis:**

The data analysis was done with MSTST. and means were compared using Multiplem Range Test.(D M R T).

## CHAPTER FOUR

### RESULTS AND DISCUSSION

The data presented in table (4-1) show the properties of the soil used in the green house experiment. The soil are clay loam in texture, alkaline , modrate in cation exchangeable capacity, high saturation percentage and low in nitrogen, organic carbon and phosphorus.

**Table 4.1: The properties of soil used in the experiment:**

ECe	pH	Soluble Cations			Soluble Anions			SAR
		Na meq/L	K meq/L	Ca+Mg meq/L	CO <sub>3</sub> meq/L	HCO <sub>3</sub> meq/L	CL meq/L	
1.4	7.7	10.7	0.3	7.8	0.0	3.2	3.6	6

Soil particles distribution			Textural Class	CEC Coml/Kg Soil	Moisture Content	Saturation %
Sandy%	Silt%	Clay%				
11	34	55	Clay Loam	55	3	80

Exchangeable Cations			N %	O.C %	P <sub>ppm</sub>	CaCO <sub>3</sub>
Na meg/100g Soil	K meg/100g Soil	Ca+Mg meg/100g Soil				
4.3	0.1	50.6	0.04	0.1	3.0	0.1

The data in table (4-2) show the properties of compost used in the experiment.

**Table 4.2: The properties of compost**

<b>pH</b>	<b>N%</b>	<b>P%</b>	<b>K%</b>	<b>C\N</b>	<b>O.C</b>
8.0	5.0	0.00047	73.4	0.43	2.13

### **1- Laboratory experiment:**

Rhizobium were cultured on yeast manitol Agar medium(YMA), This medium is selective and suitable for growth of Rhizobium.

Data in Table (4-3)shows the effect of Temperature on the Enumeration of Rhizobium.

There were no significant difference, The highest growth of Rhizobium occurred at 30° for Enrri 24 and the least growth at 40c with dilutions (10<sup>4</sup>), (10<sup>8</sup>) 1. Results explained that the optimum temperature for Rhizobium is 30c .That is agree with (Zahran,1999).

**Table 4 .3: Effect of Temperature on the Enumeration of Rhizobium ENRRI 24**

<b>Rhizobium Spp</b>	<b>20c°</b>		<b>30c°</b>		<b>40c°</b>	
	<b>10<sup>4</sup></b>	<b>10<sup>8</sup></b>	<b>10<sup>4</sup></b>	<b>10<sup>8</sup></b>	<b>10<sup>4</sup></b>	<b>10<sup>8</sup></b>
<b>Dillution</b>						
CFU	320.00 <sup>A</sup>	50.00 <sup>C</sup>	320.00 <sup>A</sup>	320.00 <sup>A</sup>	2.00 <sup>C</sup>	0.00 <sup>C</sup>
LSD	57.95	57.95	57.95	57.95	57.95	57.95

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*

Data in Table (4-4)shows the Effect of PH on the Enumeration of Rhizobium.

The pH of Rhizobium growth In Enrri24 is highe st responsive at PH<sub>6</sub>, PH<sub>7</sub>, This clearly indicates the suitability of neutral PH to the growth of Rhizobium, That is agree with (Hungria ,and Vargas 2000). also explained the effect Acidity on Rhizobium growth. are favorable for Rhizobium growth. That is agree with(Grham,etal .1994).

However Rhizobium growth was less when PH<sub>9</sub>. However it is found That in PH<sub>9</sub> Rhizobium growth was less.

**Table 4.4: Effect of PH on the Enumeration of Rhizobium.**

Rhizobium Spp	pH6		pH7		pH8		pH9	
	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>
Dilution	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>
CFU	320.00 <sup>A</sup>	320.00 <sup>A</sup>	320.00 <sup>A</sup>	320.00 <sup>A</sup>	181.00 <sup>B</sup>	185.00 <sup>B</sup>	2.50 <sup>C</sup>	2.50 <sup>C</sup>
LSD	65.63	65.63	65.63	65.63	65.63	65.63	65.63	65.63

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*

The data in Table(4-5)showed that the effect of salinity on Enumeration of Rhizobium. There are no significant different in Enri24, The rate of Rhizobium growth is varied according to different Salt concentrations. The maximum rate of growth was obtained at 0.2 and 0.4%Nacl with dilution (10<sup>4</sup>, 10<sup>8</sup>) CFU/MI However we find a few or not growth of Rhizobium was obsered at 0.6,0.8,and 1,2%Nacl with dilutions (10<sup>4</sup>, 10<sup>8</sup>) CFU/MI

There for when the concentration of salts was increase the growth of Rhizobium was slow.

**Table 4.5: Effect of salinity on Enumeration of Rhizobium**

<b>Rhizobium Spp</b>	<b>0.2 (g/L)</b>		<b>0.4(g/L)</b>		<b>0.6 (g/L)</b>		<b>0.8 (g/L)</b>		<b>1 (g/L)</b>		<b>2 (g/L)</b>	
Dilution	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>	10 <sup>4</sup>	10 <sup>8</sup>
CFU	320.00 <sup>A</sup>	320.00 <sup>A</sup>	320.00 <sup>A</sup>	320.00 <sup>A</sup>	20.00 <sup>C</sup>	0.00 <sup>D</sup>	2.50 <sup>D</sup>	0.00 <sup>D</sup>	0.00 <sup>D</sup>	1.00 <sup>D</sup>	0.50 <sup>D</sup>	0.00 <sup>D</sup>
LSD	14.89	14.89	14.89	14.89	14.89	14.89	14.89	14.89	14.89	14.89	14.89	14.89

\*Means with the same letters within the same column are not significantly different at the 0.5 level of probability by the Duncan Multiple Range Test\*

## **2- Green house experiment: Effect of treatments on plant growth:-**

The obtained results in Table (4-6) show that the Effect of organic, Rhizobium inoculation, and chemical fertilizer on plant component after 4weeks. There were no significant differences between all treatments. The data gave higher length of plant at compost 0.8 and 1.6. However the lower results were obtained at Nitrogen ( $N_1$ ) and Rhizobium  $10^4$ .

for shoot dry weight the treatments gave lower values compared to control, and for root dry weight a higher value was obtained at compost 1.6,

However the other results are similar. Number of nodules in main basic and lateral roots were very few except in Rhizobium  $10^4$ cfu,  $10^8$ cfu in basic nodules were one number, and  $10^8$  in lateral nodules were 0.001 number.

**Table 4.6: Effect of Compost, Rhizobium inoculation and Urea fertilizer on peanut plant(4WAP)**

Treatments	Plant Length (cm)	Plant color	Shoot dry weight (gm)	Root dry weight (gm)	Nodules Number Basic	Nodules Number Latral	Nodules Weight Basic	Nodules Weight Lattaral
Control	28.0 <sup>AB</sup>	4.00	2.200 <sup>A</sup>	0.100 <sup>B</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Compost 0.8	29.0 <sup>A</sup>	3.00	1.200 <sup>D</sup>	0.100 <sup>B</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Compost 1.6	27.0 <sup>AB</sup>	3.00	1.200 <sup>D</sup>	0.200 <sup>A</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Compost 3	25.0 <sup>BC</sup>	3.00	1.300 <sup>CD</sup>	0.100 <sup>B</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Nitrogen N <sub>1</sub>	14.0 <sup>D</sup>	2.00	0.300 <sup>E</sup>	0.100 <sup>B</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Nitrogen N <sub>2</sub>	25.0 <sup>BC</sup>	4.00	1.667 <sup>B</sup>	0.100 <sup>B</sup>	0.00 <sup>C</sup>	0.00	0.00	0.00
Rhizobium 10 <sup>4</sup>	23.0 <sup>C</sup>	4.00	1.600 <sup>BC</sup>	0.100 <sup>B</sup>	10.00 <sup>A</sup>	1.00	0.001	0.00
Rhizobium 10 <sup>8</sup>	17.0 <sup>D</sup>	3.00	1.000 <sup>D</sup>	0.100 <sup>B</sup>	2.00 <sup>B</sup>	1.00	0.00	0.001
LSD	3.831		0.3323	0.09592	0.4104	0.3136	-	-

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*

The data in table (4-7) showed that the effect of total NPK% on peanut plant (4WAP)

There were significant differences in nitrogen content the highest value was obtained Rhizobium  $10^8$ , and medium values are compost 1.6, 3,  $N_1$  and the lower values were obtained by control, Rhizobium  $10^4$  and compost 0.8.

In the phosphurs there were no significant differences, the higher values were obtained by Rhizobium ( $10^8$ ),

In potassium there were significant differences, the highest values was obtained by Rhizobium  $10^8$ , However the lower values was obtained by Rhizobium  $10^4$  inoculation.

**Table 4.7: The effect of NPK% on peanut plant(4WAP):**

Treatments	N%	P%	K%
Control	1.420 <sup>D</sup>	0.015 <sup>B</sup>	17.360 <sup>E</sup>
Compost 0.8	0.434 <sup>F</sup>	0.035 <sup>B</sup>	16.320 <sup>E</sup>
Compost 1.6	2.213 <sup>B</sup>	0.055 <sup>AB</sup>	23.480 <sup>C</sup>
Compost 3	1.540 <sup>CD</sup>	0.032 <sup>B</sup>	12.900 <sup>F</sup>
Nitrogen $N_1$	1.560 <sup>C</sup>	0.052 <sup>B</sup>	20.900 <sup>D</sup>
Nitrogen $N_2$	1.290 <sup>E</sup>	0.007 <sup>B</sup>	29.820 <sup>B</sup>
Rhizobium $10^4$	1.220 <sup>E</sup>	0.050 <sup>B</sup>	21.210 <sup>D</sup>
Rhizobium $10^8$	2.820 <sup>A</sup>	0.109 <sup>A</sup>	39.060 <sup>A</sup>
LSD	0.1238	0.05538	1.712

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*



The data in table(4-8)show that the Effect of organic fertilizer, Rhizobium inoculation, and chemical fertilizer on peanut plant at (8WAP)the highest length of plant was obtained by compost 0.8 and the lowest length were obtained by at compost 3 and  $N_1$ .In shoot dry weight compost1.6 gave the highest weight of compared with others. Root dry weight of all treatments gave higher values weight.

**Table 4.8: Effect of Compost, Rhizobium inoculation and Urea fertilizer on peanut plant (8WAP)**

Treatments	Plant Length (cm)	Plant color	Shoot dry weight (gm)	Root dry weight (gm)	Nodules Number Basic	Nodules Number Latral	Nodules Weight Basic	Nodules Weight Lattaral
Control	29.00 <sup>B</sup>	4.0	1.50 <sup>C</sup>	0.100 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Compost 0.8	32.00 <sup>A</sup>	3.0	1.90 <sup>B</sup>	0.133 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Compost 1.6	28.667 <sup>B</sup>	2.0	3.10 <sup>A</sup>	0.200 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Compost 3	24.00 <sup>C</sup>	2.0	0.800 <sup>D</sup>	0.100 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Nitrogen N <sub>1</sub>	24.00 <sup>C</sup>	4.0	1.30 <sup>C</sup>	0.200 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Nitrogen N <sub>2</sub>	27.00 <sup>B</sup>	4.0	1.40 <sup>C</sup>	0.200 <sup>A</sup>	0.00 <sup>C</sup>	0.00 <sup>C</sup>	0.00	0.00
Rhizobium 10 <sup>4</sup>	27.00 <sup>B</sup>	4.0	0.60 <sup>D</sup>	0.200 <sup>A</sup>	7.00 <sup>B</sup>	3.00 <sup>B</sup>	0.03	0.00
Rhizobium 10 <sup>8</sup>	27.00 <sup>B</sup>	4.0	0.60 <sup>D</sup>	0.100 <sup>A</sup>	9.00 <sup>A</sup>	5.00 <sup>A</sup>	0.02	0.002
LSD	2.321	-	0.2283	0.1108	0.9365	0.8101	-	-

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*

The data in table 4-9 showed the effect of total NPK% on peanut plant (8WAP):-The data in table 4-9 show that the effect of NPK% on pea nut plant at(8WAP)in nitrogen we obtained highest result at compost 0.8 and lowest result was obtained at nitrogen(N<sub>1</sub>) and nitrogen(N<sub>2</sub>).in poshour the gave highest result to compare with other.How ever in potasum we find highest result at to compare with other.

**Table 4.9: The effect of NPK% on peanut plant(8WAP)**

Treatments	N%	P%	K%
Control	1.027 <sup>E</sup>	0.002	9.780 <sup>G</sup>
Compost 0.8	1.750 <sup>A</sup>	0.030	45.580 <sup>A</sup>
Compost 1.6	1.520 <sup>B</sup>	0.019	27.743 <sup>C</sup>
Compost 3	1.150 <sup>DE</sup>	0.020	16.660 <sup>F</sup>
Nitrogen N <sub>1</sub>	0.400 <sup>F</sup>	0.032	20.830 <sup>E</sup>
Nitrogen N <sub>2</sub>	0.380 <sup>F</sup>	0.009	5.260 <sup>H</sup>
Rhizobium 10 <sup>4</sup>	1.420 <sup>BC</sup>	0.005	24.943 <sup>D</sup>
Rhizobium 10 <sup>8</sup>	1.260 <sup>CD</sup>	0.026	45.040 <sup>B</sup>
LSD	0.2145	-	0.05538

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplem Range Test\*

The data in table (4-10) explains the effect of organic fertilizer, Rhizobium inoculation, and chemical fertilizer at 12WAP. The data gave higher plant length is at Rhizobium  $10^8$  with compare to other. In shoot dry weight Rhizobium  $10^8$  was higher weight to compare with other, in root dry weight all treatments were medium except Rhizobium  $10^8$  was higher.

Number of nodules in main basic and lateral roots Rhizobium  $10^4, 10^8$  were highest number compare to others. Weight of nodules in main basic and lateral roots all treatments were very a few except at Rhizobium  $10^4, 10^8$  at basic were 0.055, 0.705. and the lateral were 0.060, 0.827 respectively .

**Table 4.10: Effect of Compost, Rhizobium inoculation and Urea fertilizer on peanut plant(12WAP)**

Treatments	Plant length (cm)	Plant color	Shoot dry Weight (gm)	Root dry Weight (gm)	Nodules Number Basic	Nodules Number Latral	Nodules Weight Basic	Nodules Weight Latral
Control	26.0 <sup>CD</sup>	3.00	1.00 <sup>CD</sup>	0.200 <sup>B</sup>	0.00 <sup>B</sup>	0.00 <sup>B</sup>	0.00	0.00
Compost 0.8	30.0 <sup>C</sup>	2.00	1.20 <sup>CD</sup>	0.100 <sup>B</sup>	0.00 <sup>B</sup>	0.00 <sup>B</sup>	0.00	0.00
Compost 1.6	36.0 <sup>B</sup>	4.00	4.10 <sup>B</sup>	0.100 <sup>B</sup>	0.00 <sup>B</sup>	0.00 <sup>B</sup>	0.00	0.00
Compost 3	30.0 <sup>C</sup>	3.00	25.0 <sup>CD</sup>	2.00 <sup>B</sup>	1.50 <sup>C</sup>	0.100 <sup>B</sup>	0.00	0.00
Nitrogen N <sub>1</sub>	21.0 <sup>D</sup>	3.00	0.733 <sup>D</sup>	0.100 <sup>B</sup>	0.00 <sup>B</sup>	0.00 <sup>B</sup>	0.00	0.00
Nitrogen N <sub>2</sub>	30.0 <sup>C</sup>	3.00	1.50 <sup>C</sup>	0.167 <sup>B</sup>	0.00 <sup>B</sup>	0.00 <sup>B</sup>	0.00	0.00
Rhizobium 10 <sup>4</sup>	25.0 <sup>CD</sup>	2.00	1.50 <sup>C</sup>	1.500 <sup>B</sup>	5.00 <sup>A</sup>	3.00 <sup>A</sup>	0.055	0.060
Rhizobium 10 <sup>8</sup>	42.0 <sup>A</sup>	4.00	21.80 <sup>A</sup>	0.467 <sup>A</sup>	5.00 <sup>A</sup>	3.00 <sup>A</sup>	0.705	0.827
LSD	5.035	-	0.5782	0.1356	1.303	1.303	-	-

\*means with the same letters with in the same column are not significantly different at the 0.5 level of porobability by the Duncan Multiplern Range Test\*

In nitrogen we find significant different the compost 0.8 gave higher values compared to the other, However in phosphorus we find compost 1.6 is higher and control is lower. In potassium there are significant different control is higher compared to the others .

**Table 4.11: The effect of total NPK% on peanut plant(12WAP):**

Treatments	N%	P%	K%
Control	1.340 <sup>CD</sup>	0.044	35.607 <sup>A</sup>
Compost 0.8	9.730 <sup>A</sup>	0.020	27.940 <sup>B</sup>
Compost 1.6	8.653 <sup>B</sup>	0.012	12.850 <sup>G</sup>
Compost 3	1.100 <sup>CDE</sup>	0.020	23.180 <sup>D</sup>
Nitrogen N <sub>1</sub>	1.440 <sup>C</sup>	0.034	19.000 <sup>F</sup>
Nitrogen N <sub>2</sub>	1.480 <sup>C</sup>	0.022	24.380 <sup>C</sup>
Rhizobium 10 <sup>4</sup>	0.960 <sup>DE</sup>	0.023	18.470 <sup>F</sup>
Rhizobium 10 <sup>8</sup>	0.760 <sup>E</sup>	0.015	22.440 <sup>E</sup>
LSD	0.4465	-	0.5939

\*means with the same letters within the same column are not significantly different at the 0.5 level of probability by the Duncan Multiple Range Test\*

# CHAPTER FIVE

## CONCLUSION AND RECOMMENDATION

### 5.1 Conclusion:

The use of Compost and Rhizobium inoculation gave positive impact of growth components of peanut in the green house. Significant at compared to urea.

### 5.2 Recommendations:

- 1- Peanut growers advised to used compost and Rhizobium inoculation instead of chemical fertilizers to increase the growth of their plants.
- 2- The compost and Rhizobium inoculation are safe for the environment and cheap in cost compared to the chemical fertilizer.

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## Appendices



**Plate (1): Rhizobium spp.**



**Plate (2): Media of Rhizobium**



**Plate (3): Nodulated Roots Peanut**



**Plate (4): Compost**





**Plate (5): Inoculum of Rhizobium (ENRI 24)**