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Sudan University of Sciences and Technology

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EFFECTS of BIOFERTILIZATION on GROWTH, YIELD COMPONENTS and YIELD of SWEETPOTATO (*Ipomoea batatas* L.)

تأثير التسميد الإحيائى على نمو ومكونات إنتاج و انتاجية محصول البطاطا الحلوه" البامبى"

A Thesis submitted to Sudan University of Sciences and Technology in fulfillment of the requirements for the Degree of Doctor of Philosophy in Soil Science (Soil Microbiology and biofertilizers).

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{24} فَلْيَنظُرِ الْإِنسَانُ إِلَى طَعَامِهِ {25} أَنَّا صَبَبْنَا
الْمَاءَ صَبًّا {26} ثُمَّ شَقَقْنَا الْأَرْضَ شَقًّا {26}
فَأَنبَتْنَا فِيهَا حَبًّا {27} وَعِنَبًا وَقَضْبًا {28} وَزَيْتُونًا
وَنَخْلًا {29} وَحَدَائِقَ غُلْبًا {30} وَفَاكِهَةً وَأَبًّا {31}
مَّتَاعًا لَّكُمْ وَلِأَنْعَامِكُمْ {32}

صدق الله العظيم

سورة عبس الآيات (24-32)

Dedication

This work is dedicated To

My beloved young daughter ***NadaYassin,*** *who missed me for long time during this research work, and her youngest sister* ***Talia Yassin.***

To: My father who did a lot for our success.

To: My small family members:

Tahani Yassin

Abdul khalig Yassin

Ammar Yassin

Tasabeeh Yassin

Safaa Yassin

Thanaa Yassin

and the patient partner my wife ***Zahra Taha Ahmed.***

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Abstract

A pot experiment and tow field experiments were conducted to study the effect of Al Khaseeb organic fertilizer application rate and *Azospirillum brasilense* inoculation on growth and growth components of, sweetpotato (*Ipomoea batatas* L). *Azospirillum brasilense* inoculum applied with intensity of; (0, 10^4 and 10^8Cfu/ml), respectively and

the three rates of Al Khaseeb Organic fertilizer applied were; (0 t/ha, 25 t/ha and 50 t/ha). The experiment was set in a factorial arrangement in a Completely Randomized Design (CRD) with three replicates. application of Al Khaseeb at the rate of 25t/ha+ AzospiriIIum 10^4 showed significant effect ($P < 0.05$) on shoot dry weight, shoot fresh weight and root length with (5.10g), (26.70 g) and (41.33cm) compared to (1.3g), (3.67g) and (16.67 cm) for uninoculated and unfertilized control. inoculation with AzospiriIIum 10^4 +25 t/ha of Al Khaseeb organic fertilizer showed results similar to application of 50 t/ha of Al Khaseeb organic fertilizer alone, The results indicated the great potential of using organic fertilizers and biofertilizers to improve growth of sweetpotato and 50% of Al Khaseeb organic fertilizer requirement can be saved by inoculation with AzospiriIIum 10^4. .

Tow field experiments were conducted in the Experimental Farm of the College of Agricultural Studies, Sudan University of Sciences and Technology-Shambat and the Experimental Farm of the Faculty of Agricultural and Environmental Sciences, University of Gadarif- Tawawa, to study the effect of inoculation with *Azospirillum brasilense, Flavobacterium spp,* and Al Khaseeb organic fertilizer application rate on growth, yield components and yield of sweetpotato (*Ipomoea batatas* L). *Azospirillum brasilense* and *Flavobacterium spp* inoculum were applied at the concentration of (10^8Cfu/ml), with three rates of Al Khaseeb organic fertilizer; (0 t/ha, 0.8 t/ha and 1.2 t/ha). The experiments were set in a factorial arrangement in a Randomized Complete Block Design (RCBD) with three replicates. Application of *(Azospirillum* +1.2 t/ha of Al Khaseeb organic fertilizer) and (*Azospirillum* + *Flavobacterium*+ 0.8 t/ha of organic fertilizer) showed significant effect ($P < 0.05$) on sweetpotato stem length, Leaf number, branches number six and sixteen weeks after planting; with (107.67cm), (72) and(5); (193cm), (193) and(13) respectively compared to non-inoculated and non-fertilized control, also application of *Azospirillum* + *Flavobacterium*+ 0.8 t/ha of organic fertilizer showed a significant effect ($P < 0.05$) on marketable storage roots yield with (13.5 t/ha) compared to (6.71 t/ha) for the non-inoculated and non-fertilized control. However, the application of 1.2 t/ha of Al Khaseeb organic fertilizer alone showed lowest values in Leaf number, branches number in Shambat site. However in site 2 Tawawa(University of Gadarif) stem length at six weeks after planting and stem length, leaf number and branches number at sixteen weeks after planting, showed significant difference ($P < 0.05$) among treatment means; with (127.1), (270.8),(256)

and (12), compared to; (88.6), (159.2), (143) and (6) for non-inoculated and non-fertilized control respectively. The results indicated the great potential of combined application of *Azospirillum brasilense*, *Flavobacterium* biofertilizers and Al Khaseeb organic fertilizer in improving growth, yield components and yield of sweetpotato under field conditions.

مستخلص البحث

أجريت تجربة أصيص وتجربتين حقليتين لدراسة تأثيرجرعة السماد العضوي (الخصيب) والتلقيح بالسماد الإحيائي ببكتريا الأزوسبيريليم على نمو ومكونات إنتاج وانتاجية محصول البطاطا الحلوة(البامبى). تم تطبيق ثلاثة مستويات من لقاح السماد الإحيائي ببكتريا الأزوسبيريليم وكانت :(صفر و 10^4 و 10^8) cfu وحدة مكونة للمستعمرة في الملليترمن اللقاح على الترتيب كما تم إستخدام ثلاث جرعات من السماد العضوي"الخصيب" :صفر و25 طن للهكتار و 50 طن للهكتار، تم تصميم التجربة بنظام التصميم العشوائي الكامل (CRD) وبثلاث مكررات. وأظهرت النتائج تأثيرات ذات دلالة معنوية عند ($P < 0.05$)على الوزن الجاف والوزن الرطب للسيقان والأوراق وطـــول الجـــذورعند التلقيح بالسماد الإحيائي ببكتريا الأزوسبيريليم10^4 + 25 طن للهكتار من السمإد العضوي" الخصيب" اذ أعطـت (5.10 g) و (26.70g) و (41.33 cm) بالمقارنة مع (1.39 g) و (3.67g) و (16.67 cm) لمعاملات الشاهد التى لم يتم تلقيحها أو تسميدها., والتلقيح ببكتريا الأزوسبيريليم10^4 + 25 طن للهكتار من السمإد العضوي " الخصيب" اعطت نتائج مشابهة للتطبيق 50 طن للهكتارمن السمإد العضوي" الخصيب" لوحده، وتشير هذه النتائج لأهمية استخدام التلقيح ببكتريا الأزوسبيريليم10^4 مع السمإد العضوي" الخصيب" ويمكن توفير 50% من احتياج السمإد العضوي "الخصيب"من خلال التلقيح ببكتريا الأزوسبيريليم بكثافة 10^4.

كما أجريت تجربتين حقليتين بكل من المزرعة التجريبية لكلية الدراسات الزراعية، جامعة السودان للعلوم والتكنولوجيا(شمبات)، والمزرعة التجريبية لكلية العلوم الزراعية والبيئية - جامعة القضارف (تواوا)، لدراسة تاثيرالتلقيح ببيكتريا الأزوسبيريليم وبكتريا الفلافو باكتيريم مع معدلات مختلفة من السماد العضوى"الخصيب"على نمو ومكونات الإنتاج وإنتاجية محصول البطاطا الحلوة "البامبي" تحت ظروف الحقل. إذ تم التلقيح بتركيز cfu 10^8 وحدة مكونة للمستعمرة من نوعى السماد الحيوي بالإضافة إلى ثلاث معدلات من السماد العضوي"الخصيب": صفر و0.8 طن للهكتار و1.2 طن للهكتاروتم تصميم التجربة بنظام القطاعات العشوائية الكاملة (RCBD) وبثلاث مكررات. أظهرت النتائج وجود تاثيرات ذات دلالة معنوية على طول السيقان وعدد الأوراق فى فترات 6 و16 أسبوع بعد الزراعة عند التلقيح ببكتريا الأزوسبيريليم+ 1.2 طن للهكتارمن السماد العضوي الخصيب، يليه التلقيح بنوعى السماد الحيوي+ 0.8 طن للهكتارمن السماد العضوي، اذ أعطت قيم:(107.67سم) و(72)و (5): و(193سم) و(193) و (13) على الترتيب بالمقارنة مع معاملات الشاهد التى لم تلقح ولم تسمد. كما أظهرت النتائج ان التلقيح المزدوج بنوعي السماد الإحيائي +0.8 طن للهكتار من السماد العضوي "الخصيب" له

تأثير ذو دلالة معنوية ($P < 0.05$)على تكوين جذورالبامبي القابلة للتسويق بإنتاج (13.5) طن للهكتار مقارنة مع (6.71) طن للهكتار لمعاملات الشاهد التي لم تلقح ولـم تسـمد. كما لوحظ أن التسميد بالجرعة الكبيرة للسماد العضوي 1.2 طن للهكتار لوحده وبدون التلقيح بالسماد الإحيائي أعطى أقل القيم في عدد الأوراق وعدد الفروع في موقع شمبات بالتجربة الحقلية الأولى. بينما في الموقع 2 تواوا (بجامعة القضارف) وجد أن طول السيقان بعد 6 اسابيع من الزراعة وطول السيقان، وعدد الأوراق وعدد الفروع بعد 16 اسبوع اظهرت فروق ذات دلالة معنوية بين متوسطات المعاملات حيث اعطت:(127.1)، (270.8)، (256) و(12) بالمقارنة مع(88.6)، (159.2)، (143) و(6) على الترتيب لمعاملات الشا هد التى لم تلقح ولم تسمد.

دلت النتائج على أهمية التلقيح المزدوج بنوعي السماد الاحيائي مع جرعة معقولة من السماد العضوي "الخصيب" لتحسين نموواإنتاج البطاطا الحلوة (البامبي) مع تخفيض تكلفة إنتاجه بالإضافة لتحسين خصائص المنتج تحت ظروف الحقل.

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ANOVA	: Analysis of Variance.
ARS	:Agricultural Research Services
CIP	: International Potato Center.
CRD	: Completely Randomized Design.
DMRT	: Duncan's Multiple Range Test.
IU	: International Unit
FYM	:Farm Yard Manure
LSD	: Least Significant Difference.
MOP	: Muriate of Potash.
PGPR	: Plant Growth Promoting Rhizobacteria.
PSB	: Phosphate Solubilizing Bacteria.
R/S	: Root to shoot ratio.
RCBD	: Randomized Complete Block Design.
TSP	: Triple Super Phosphate.
VAM	:Vesicular Arbuscular Mycorrhiza.
WAP	: Weeks After Planting.

CHAPTER ONE

INTRODUCTION

Sweetpotato represents a potential food crop that can help millions of people in food supplies worldwide. It is getting more attention as one of the most important food crops that can help in solving the global problem of fast growth of population and shortage in food crops production. Also it can improve the nutritional value of other foods if mixed with them, as it contains a considerable amount of provitamin A and other vitamins such as vitamin C (ascorbic acid) and minerals. Sudan has all the favorable conditions for sweetpotato production, and orange fleshed sweetpotato cultivars were introduced earlier in Sudan, but were lacking the consumer preference. More efforts are needed to enable this crop to play important roles in social and economical development (Ahmed, 1987).

Sweetpotato *Ipomoea batatas* (L.) is ranked the fifth among the world most important food crops, with more than 133 million tonnes of annual production (CIP, 2005). It is a tuberous - rooted perennial mainly grown as annual. The area under cultivation was 8.5 million ha in 2009 and average worldwide tuber yield was 12648 kg/ha.

Sweetpotato is one of the seven world food crops with annual production of more than 100 million metric tons. Average storage roots yield are 5t/ha in Africa, 10 t/ha in south Americas and16t/ha in Asia. The main sweetpotato producers are China, Indonesia, Vietnam, India, Philippines, and Japan in Asia, Brazil and the USA in the Americas and Nigeria, Uganda, Tanzania, Rwanda, Burundi, Madagascar, Angola and Mozambique in Africa (FAO, 2010).

It is necessary to increase the production of sweetpotato as a potential food crop that can play an important role in bridging the gap between the demand and production of food if it's exploited properly. The use of mineral fertilizers, herbicides and pesticides to increase or enhance the production of sweetpotato leads to environmental problems and pollution in water resources through the run off of the leached chemicals. So the trend is to find scientific method that keeps the balance, between the high production of sweetpotato and other strategic food crops and conservation of environment and the natural resources. To fulfill such formulation, we have to use organic and biofertilizers to replace the chemical fertilizers totally or partially and sustain the production of important crops such as sweetpotato.

Biofertilization is the addition of biofertilizers to replace the chemical fertilizers totally or partially. They include different kinds of micro-organisms such as phosphate solubilizing bacteria (PSB), symbiotic micro-organisms such as (*Rhizobium, Bradyrhizobium* etc.) and non-symbiotic micro-organisms such as (*Pseudomonas*, *Azospirillum* and *Azotobacter*). In addition to mycorrhizal fungi association. The use of biofertilizers in enhancing plant growth and yield has gained great attention in recent years because of chemical fertilizers high cost and hazardous effect on the environment (Ghazi, 2006).

Bio-fertilizers can be applied to reduce the production cost and to combat or suppress soil borne pathogens. Biofertilizers application for fruit crops has become in the last few years a positive alternative to chemical fertilizers.

Currently no research has been done on integrated use of combined organic and biofertilizers application pertaining to storage roots yield components and yield of sweetpotato. In view of this fact, a systematic investigation for the effect of using organic fertilizers like AI Khaseeb and locally available, accessible and affordable biofertilizers for increasing yield components and

yield of sweetpotato. To address those problems, the study was initiated with the objectives of investigating the effect of AI Khaseeb organic fertilizers and *Azospirillum brasilense* and *Flavobacterium* spp. biofertilizer application on the yield components and yield of sweetpotato under field conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sweetpotato

Sweetpotato is the second most important root crop ranked after cassava (*Manihota esculent* Crantz) grown mainly as a cash crop or subsistence crop, in small farm or beside rivers or in gardens for the fresh food market or home consumption. Sweetpotato is a major crop that feeds millions of people in developing world. It is especially popular among farmers with limited resources, and produces more biomass and nutrients per hectare than any other food crop in the world (Prakash, 1994). It is adaptable to a broad range of agro-ecological conditions and fits in low input agriculture. It is in many ways an ideal crop for farmers as it grows on low nitrogen soils, tolerates drought well, crowds out weeds, suffers relatively from few pests, and is highly productive even under adverse farming conditions. It is grown in more than 100 countries as a valuable source of food, animal feed and industrial raw material. It is a staple food in most South East Asian and African countries (Onwueme, 1978). China is the largest producer of sweetpotato with about 80% of world production. Among the food crops, sweetpotato has the highest recorded net protein utilization "based on percentage of food nitrogen retained in the body" in addition, the orange fleshed sweetpotato are rich in ß-carotene, a nutrient which may be effective in preventing certain types of cancer (Prakash, 1994).

2.1.1 Climate

The plant grows best at an average temperature of 24^{o}C, abundant sunshine and warm nights, annual rainfalls of 750-1000 mm are considered most suitable, with minimum of 500 mm in the growing season. The crop is

sensitive to drought at the stage of tuber initiation (50-60)days after planting and it is not tolerant to water logging as it may cause tubers rots and reduce growth of storage root (Ahn,1993). Sweetpotato is cultivated throughout tropical and warm temperate regions, wherever there is sufficient water to support their growth (Stephen and O'Hair 1990). Day-length affects both the flowering and the storage root-formation process, Day-length of 11 hours or less promotes flowering and at day-lengths greater than 13.5 hours flowering fails to occur, in the tropics sweetpotato flowers frequently. The storage roots formation is also promoted by short-day conditions. Short day with low light intensity promote storage roots formation, while long day tend to favor vine development at the expense of the storage roots formation. Sweetpotato grows best on sandy-loam soils and does poorly on clay soils. In general sandy soils are more suitable for sweetpotato planting. However for highly sandy soils a higher fertilization rate of organic matter is required (Saad, 1994).

Good drainage is essential since the crop cannot withstand water logging. The preferred soil pH range for sweetpotato is 5.6-6.6 and it is sensitive to alkaline or saline soils (Onwueme, 1978).

2.1.2 Growth cycle

Sweetpotato is a perennial plant but grown as an annual. It is usually propagated from vine cuttings. The growth of such plants occurs in three more or less distinct phases a) an initial phase when the fibrous roots grow extensively and there is only moderate growth of the vines, b) a middle phase when the vines make extensive growth and the storage roots are initiated - a tremendous increase in the leaf area occurs during this phase; and c) a final phase when storage roots bulking occurs and very little further growth of vines and fibrous roots begins to decline (Onwueme, 1978).

2.1.3 The root system

When sweetpotato is planted from stem cuttings in normal cultivation, adventitious roots arise from the cutting in a day or two. These roots grow rapidly and form the fibrous roots, which absorb nutrients and water and anchor the plant. Such roots grow into the soil and greatly increase the effective feeding area of the plant. Storage roots are the lateral roots which store the photosynthetic products, and it's the commercial parts of sweetpotato. As the plant matures, thick pencil roots that have some lignifications are formed (Huamán, 1997).

2.1.4 Storage roots

Sweetpotato produces storage roots that develop as a result of the secondary growth of a few roots within the top 20-25 cm of soil. Most of the storage roots develop from the initial fibrous root system of the plant. The process of secondary growth leads to storage roots formation (Onwueme, 1978).

2.1.5 Factors affecting storage roots formation

Several environmental factors affect storage roots formation in sweetpotato. Light exerts its influence in two major ways. Firstly, storage roots formation in sweetpotato is promoted by short day-lengths and retarded by long day-length. Secondly, normal growth and development of the sweetpotato storage roots can only occur in the absence of light. Exposure of the root system to light prevents any of the roots from forming storage roots. The oxygen content of the soil is another factor which influences storage roots formation in sweetpotato. Inadequate oxygen results in retardation of storage roots formation and this partly accounts for the poor performance of sweetpotato on water logged soils or soils of high bulk density. Excessive nitrogen fertilizer in the soil delays storage roots formation, while low night temperature promotes it, morphologically the mature sweetpotato storage roots may range

in shape from spherical to nearly cylindrical or spindle-shape, in size from 0.1 kg to over 1kg, and in length from a few centimeters to over 30 cm (Onwueme, 1978).

2.1.6 Shoot system

Stems of sweetpotato are cylindrical and it's length depend on growth habit of the cultivar (erect, semi erect, spreading and very spreading, and water availability in the soil (Huamán, 1997). Stem length varies with cultivars, and may range from about 1 m to over 6 m. Leaves are simple and may have entire margins. The lamina is green in colour, sometimes with purple coloration especially along the veins. Stomata are present on both the upper and lower leaf surfaces, but are more numerous on the lower surface. Sweetpotato flower has five united sepals and five petals joined together to form a funnel-shape corolla tube. This tube is purplish in colour and is the most conspicuous part of the flower. The stamens are five in number and are attached to the base of corolla. The filament is white and hairy and the anther is also white and contains numerous rounded pollen grains, which bear minute papillae on their surfaces. The ovary consists of two carpel each of which contains one locule. Each locule contains two ovules, so that there is a maximum of four ovules in each ovary. Each flower when mature opens before dawn on a particular day. It stays open for only a few hours then closes and welts before noon on the same day. Pollination is by insects particularly bees (Onwueme, 1978).

2.1.7 Tillage and seedbed preparation

Sweetpotato is grown on ridges, mounds and on the flat land. Cultivation on mounds gives good yields and is extensively practiced. Planting on ridges is the most universally recommended method of growing sweetpotato. The growing of sweetpotato on the flat should be discouraged because the

resulting yields are usually low (Onwueme, 1978). Land preparation on mineral soils including sandy soils and drained peat involves one round of disc ploughing followed by one round of harrowing or rotor tilling, Ridges at 1.0 m apart are then built using a tractor-mounted ridger. The optimum size of ridge is 30 cm high and 60 cm wide at the base (Tan and Saad, 1994).

2.1.8 Planting

Sweetpotato grow adequately if propagated by means of the storage roots or by means of vine cuttings. The use of vine cuttings is the recommended commercial method of propagating sweetpotato. In the use of vine cuttings, pieces from the stem apex are preferred to those from the middle and basal portions of the stem. The yields of storage roots tend to increase with increase in the length of vine cuttings used, but a length of about 30 cm is recommended (Onwueme, 1978). Vine cuttings are used in planting, the best coming from the apical portions of healthy vines. These 30 cm-long cuttings are partially buried at 25 cm apart, leaving the distal tips exposed. Plant density works out to 40000 per hectare (Tan and Saad, 1994). At planting the vine is inserted into the soil at an angle so that one-half to two thirds of its length is beneath the soil. The placement of the vines or sprouts is done by hand. The vines are normally planted 25-30 cm apart on ridges that are 60-75 cm apart. Sweetpotato is able to compensate to some extent for variations in the planting density. As plant population per hectare increases the number of storage roots per plant decreases, the mean weight per storage roots decreases and the yield per plant decrease (Onwueme, 1978).

2.1.9 Weed control

Weeds are a problem in sweetpotato only during the first two months of growth. After this period, vigorous growth of the vines causes rapid and effective coverage of the ground surface and smothers the weeds present. The

use of herbicides to control weeds in sweetpotato is widely practiced in various parts of the world (Onwueme, 1978). Weed control is best accomplished by good land preparation so that the field is free from weeds at the time of planting. Additionally, the field is sprayed with *Alachlor* at the rate of 2-4 L ha^{-1} a week before planting. This pre-emergence herbicide should keep weeds in check for 2-3 months, by which time the crop canopy would have been fully developed to shade out weeds. When it is necessary, manual weeding with a hoe is carried out at 3-4 weeks after planting (Tan and Saad, 1994).

2.1.10 Diseases

The most common disease observed on sweetpotato is scab caused by the fungus *Elsinoe batatas*. The disease affects the young shoot and vines, causing severe distortion of the unfolded leaves as well as scab-like lesions along the main veins and midrib on the underside of the leaves, petioles and vines. It may be controlled by a fungicide such as *Benomyl*, sprayed every 10-14 days (Anon, 1978; Tan and Saad, 1994).

2.1.11 Nutrients content

Besides simple starches, sweetpotato is rich in complex carbohydrates, dietary fiber and beta-carotene (a provitamin A carotenoids). In general sweetpotato varieties with dark orange flesh have more β-carotene than those with light colored flesh (Coghlan, 2012). Sweetpotato is an excellent source of flavonoid phenolic compounds such as beta-carotene and vitamin A (100 g tuber provides 14187 IU of vitamin A and 8509 µg of β-carotene). The value is one of the highest among the root-vegetables categories. These compounds are powerful natural antioxidants. Vitamin A is also required by the body to maintain integrity of healthy mucus membranes and skin. It is a vital nutrient for acuity of vision. Consumption of natural vegetables and fruits rich in

flavonoids helps to protect from lung and oral cavity cancers (Nutrition, 2015).

2.1.12 Fertilization

Fertilization is the operation of artificial addition of nutrient to soil to enhance plant growth, and maintain the nutrient level in the soil at reasonable fertility level to meet the plant nutrients requirements. The most frequently applied fertilizers to the soil are nitrogen, phosphorus and potassium. Plants need a wide range of proteins to grow, develop and mature. The main body of protein is amino acids and nitrogen (N) is the major component of amino acids. Nitrogen is also present in chlorophyll. Soil micro-organisms feed on soil N during breakdown of organic materials. Nitrogen improves quality of leafy vegetables. It promotes rapid growth and if the supply is out of balance with other nutrients flowering and fruiting may be delayed. Plant nitrogen concentration range from 2% to 6%, and plants take in N as NO_3 (nitrate) or NH_4 (ammonium). Nitrogen in the plant is used in amino acid synthesis, which forms protein and nucleic acid. Nitrogen is in chlorophyll protein and essential for success photosynthesis and it greatly affect protein content of the product.

Phosphorus is the second most deficient element. It's concentration in plant ranges from 0.1% to 0.4%. Plants absorb P mostly as orthophosphate ions H_2PO_4 and these ions are found in very low concentrations in the soil solution. The most important functions of P in plant are in energy storage and transfer reactions, also it can improve product quality; increase resistance to disease. The plants potassium concentration is in the range of 1% to 4%, K is taken in the form of potassium ion K^+, It plays a large role in enzyme activation and regulation of osmotic pull that draw water in the plant root, and

K deficiency can cause poor water use efficiency and reduce total N uptake and protein synthesis in the plant (Fertility2, 2005).

2.1.13 Yield of sweetpotato crop

In the year 2010, the world average annual yield for sweetpotato crop was 13.2 tons per hectare. The most productive farms of sweetpotato breeds were in Senegal, where the nationwide average annual yield was 33.3 tonnes per hectare (FAO, 2010).

In Sudan main sweetpotato producing areas are New Halfa Scheme, Rahad Scheme, Damazein and previous southern states; average yield is 8-15t/ha and dropped to 5–8 t/ha. The sweetpotato stands as one of the most important crops in the rapidly expanding vegetable industry of the Sudan (Ahmed, 2000).

2.2 Biofertilizers

Biofertilizer is a product that contains living microorganisms, which exert direct or indirect beneficial effects on plant growth and crop yield through different mechanisms. The term biofertilizer as used here could include products containing bacteria to control plant pathogens, but these are frequently referred to as biopesticides (Siddiqui and Mahmood, 1999; Burdman, *et al*., 2000; Vessey, 2003). Biofertilization is the addition of biofertilizer to replace the chemical fertilizer completely or partially. The use of biofertilizer in enhancing plant growth and yield has gained great attention because of chemical fertilizers high cost and hazardous effect on the environment. Combined inoculation of Vesicular Arbuscular mycorrhizal fungi (VAM) and *Azospirillum* were found to enhance the growth and production of various vegetable crops (Ghazi, 2006).

Application of biofertilizers as amendments to fruit crops reduced pollution happened concerning both soil and underground water (Maksoud, *et al*., 2009). Ashokan, *et al*., (2000) reported that a significant at ($P < 0.05$) enhancement of banana plants as a result of dual inoculation of vesicular fungi (VAM) and Azotobacter. *Rhizobium* inoculation indicates an increased faba bean seed yield, ash; fat, crude protein and 100 seed weight (Awad, *et al*., 2010).

2.2.1 Types of biofertilizers

Biofertilizer include different kinds of microorganisms such as phosphate solubilizing bacteria (PSB), symbiotic microorganisms such as (*Rhizobium*, *Bradyrhizobium* etc.) and non-symbiotic microorganisms such as (*Pseudomonas*, *Azospirillum* and *Azotobacter*). In addition to mycorrhizal fungi association (Ghazi, 2006). Now a days there are different types of biofertilizers includes *Rhizobium*, *Azotobacter*, *Azospirillum*, *Cyanobacteria*, Azolla, Phosphate Solubilizing Micro-organisms (PSM) and Arbuscular Mycorrhizal fungi (AM).

2.2.2 Application of biofertilizers

Application of biofertilizers can not only reduce chemical fertilizers use by 20 to 50 percent but also can simultaneously increase the yield of crop by 10 to 20 per cent. Among the different beneficial soil microbes, *Azospirillum* spp. helps in nitrogen fixation and it also produces some growth promoting substances like Indole Acetic Acid (IAA) and GaberIine (GA). However, the informations available is much scanty on the beneficial role of biofertilizers particularly *Azospirillum* on growth and yield of sweetpotato (Saikia and Borah, 2007).

2.2.3 Crop response

Singh, *et al.,* (2010) reported that the *Azospirillum* inoculation has been found beneficial in a variety of crops including cereals, forages and other crops. Also they reported that among various field crops sorghum and millets responded better than others under rain fed conditions.

2.2.4 Factors affecting the crops response

The crop response however varies greatly depending upon types of crop and variety, location, season, soil fertility level, native micro-organisms and interaction, etc. Crop response to *Azospirillum* inoculation is mainly attributed to its ability to fix atmospheric nitrogen and to produce Phytohormones (Singh, *et al.*, 2010).

2.3 Plant growth promoting rhizobacteria role

Plant growth promoting rhizobacteria(PGPR) and bacterial mechanisms of plant growth promotion include biological nitrogen fixation(BNF), synthesis of Phytohormones, environmental stress relief, synergism with other bacteria-plant interactions, inhibition of plant ethylene synthesis, as well as increasing availability of nutrients like phosphorus, iron and minor elements, and growth enhancement by volatile compounds (Siddiqui, 2005).

2.3.1 *Azospirillum*

Inoculation of plants with beneficial micro-organisms is one of the methods to increase the plant growth and yield. *Azospirillum* is considered the most important rhizobacterial genus involved in improvement of plant growth or crop yield worldwide (Bashan, *et al.*, 2004). Bacteria of the genus *Azospirillum* are associative nitrogen (N_2)-fixing rhizobacteria that are found in close association with plant roots. Genus *Azospirillum* (K-subclass of proteobacteria). They are able to exert beneficial effects on plant growth and

yield of many agronomic crops under a variety of environmental and soil conditions. *Azospirillum* and other bacteria were found to be a potential bio-fertilizers for sweetpotato as it increased yield (Farzana and Radziah, 2005). The optimum temperature for *Azospirillum* growth is 32-35 c^{o}, this might be the reason for better performance of inoculated crops in summer under irrigated conditions. *Azospirillum brasilense* lives in soil and it is able to live on its own in the soil, or in close association with plants in the rhizoplane (the area right next to the roots of plants in the soil). A. *brasilense* is helpful to plants and important to farmers because it is able to fix nitrogen–it can convert nitrogen gas in the air into nitrogen bound up in amino acids and proteins. *Azospirillum* is a common soil habitant of tropics (Singh, *et al*., 2010).

2.3.2 *Flavobacterium*

Flavobacterium is a genus of Gram-negative, non-motile and motile, rod-shaped bacteria that consists of 130 recognized species as well as three newly proposed species (*F. gondwanense*, F. *salegens*, and *F. scophthalmum*). *Flavobacteria* are found in soil and fresh water in variety of environments and fixes nitrogen (Wikipedia, 2016).

2.3.3 Benefits of *Azospirillum* and *Flavobacterium* inoculation:

2.3.3.1 Nitrogen fixation

Nitrogen fixation is a process in which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_4^+). Atmospheric nitrogen or molecular dinitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. The fixation process frees nitrogen atoms from their triply bonded diatomic form, N≡N, to be used in other ways (Wikipedia, 2016). *Azospirillum* and *Flavobacterium* is known to be a very active nitrogen fixers under laboratory as well as soil conditions providing fast

growth, better health of the plant and higher yield (Kannan and Ponmurugan, 2010). *Azospirillum* is considered to be important growth promotive rhizobacteria that can improve the growth and yield of several plants including economically important cereals and grasses. *Azospirillum*-plant association leads to the enhanced development and increased yield of different host plants under appropriate growth conditions (Singh, *et al.*, 2010).

2.3.3.2 Phytohormones production

Plant hormones are a group of naturally occurring substances, which at low concentrations play a crucial role in the development of plants (Davies, 1995). Soil microorganisms particularly those present in the rhizosphere soils, are potential source of Phytohormones. Phytohormones production by PGPR represents classic example of plant-microbe interaction. Phytohormones of bacterial origin benefit plant by altering plant physiology, morphology, leading to improved mineral and water absorption (Perrig, *et al.*, 2007). Costacurta and Vanderleyden (1995) reported that many beneficial bacteria produce awide range of Phytohormones (auxins, cytokinnins and gibberellins) and enzymes such as pectinase that are involved in the infection process of plant microbe symbiosis. Recently, the most common explanation for the effect of rhizobacteria on plants is based on the production of Phytohormones that alter plant metabolism and morphology, leading to improved mineral and water absorption (Perrig, *et al*., 2007).

2.3.3.3 Disease resistance

Agriculturally important grasses contain numerous diazotrophic bacteria, the interaction of which are speculated to have some other benefits to the host plants. In study conducted to analyze the effect of a bacterial endophyte, *Azospirillum* sp. B510 on disease resistance in host rice plants (*Oryza sativa* cv. *Nipponbare)*. The plants exhibited enhanced resistance against diseases

caused by virulent rice blast fungus *Magnaporthe Oryzae* and bacterial Pathogen *Xanthomonas oyzae,* in rice plants.(Yasuda, *et al.,* 2009).

2.3.3.4 Drought tolerance

Application of biofertilizers as general and *Azospirillum* specifically can help the crop to withstand drought stress as it stimulates the production of plant growth regulators or plant hormones and improves plant health, which can be a limiting factor in facing the drought stress (Perrig, *et al.*, 2007).

2.3.3.5 Reduction of production cost

The biofertilizers maintain soil quality and they are cost effective as the inputs of biofertilizers production are mostly available in the field, and it's production needs less energy compared with the production of mineral or chemical fertilizers. All this can reduce the production cost of the crops, more over it's eco-friendly and renewable source of nutrient for the crops in the soil.

2.3.4 Soil N content and *Azospirillum* biofertilizer

Soil nitrogen content affects the performance of *Azospirillum* inoculation, and increased nitrogen dose is believed to retard the inoculum performance. Single inoculation of *Azospirillum* showed 20 percent increase in yield, while combined inoculation of *Azospirillum* and *Azotobacter* showed 27.2 increases in yield (Singh, *et al.*, 2010).

2.4 Organic Fertilizers

Organic fertilizers are fertilizers derived from animal matter human excreta or vegetable matter. (e.g. compost, manure)(Dittmar, *et al.*, 2009). In contrast, the majorities of fertilizers are extracted from minerals (e.g., phosphate rock) or produced industrially (e.g., ammonia). Naturally occurring organic

fertilizers include animal wastes from meat processing, peat, manure and slurry (Wikipedia, 2015).

2.4.1 Sources of organic fertilizers

The main organic fertilizers are in ranked order: peat, animal wastes (often from slaughter houses), plant wastes from agriculture, and sewage sludge (Dittmar, *et al*., 2009).

2.4.1.1. Peat

The main source of organic fertilizer is peat, an immature precursor to coal. Peat is the most widely used organic fertilizer. Peat itself offers no nutritional value to the plants, but improves the soil by aeration and absorbing water (Wikipedia, 2015).

2.4.1.2 Animal wastes

These materials include the products of the slaughter of animals' Blood meal, bone meal, hides, hoofs, and horns are typical precursors. Chicken litter, which consists of chicken manure mixed with sawdust, is an organic fertilizer that has been shown to better soil condition for harvest than synthesized fertilizer. Researchers at the Agricultural Research Service (ARS) studied the effects of using chicken litter, as organic fertilizer, versus synthetic fertilizer on cotton fields, and found that fields fertilized with chicken litter had a 12% increase in cotton yields over fields fertilized with synthetic fertilizer. In addition to higher yields, researchers valued commercially sold chicken litter at a $17/ton premium (to a total valuation of $78/ton) over the traditional valuations of $61/ton due to value added as a soil conditioner(Wikipedia, 2015).

2.4.1.3 Plant wastes

Processed organic fertilizers include compost, humic acid, amino acids, and seaweed extracts. Other examples are natural enzyme-digested proteins, fish meal, and feather meal. Decomposing crop residue (green manure) from prior years is another source of fertility. Other ARS studies have found that algae used to capture nitrogen and phosphorus runoff from agricultural fields can not only prevent water contamination with these nutrients, but also can be used as an organic fertilizer. ARS scientists originally developed the "algal turf scrubber" to reduce nutrient runoff and increase quality of water flowing into streams, rivers, and lakes. They found that these nutrient-rich algae, once dried, can be applied to cucumber and corn seedlings and resulted in growth comparable to that seen using synthetic fertilizers (Wikipedia, 2015).

2.4.1.4 Sewage sludge

Animal sourced urea and urea-formaldehyde from urine are suitable for organic agriculture; however, synthetically produced urea is not, the common thread that can be seen through these examples is that organic agriculture attempts to define itself through minimal processing (Wikipedia, 2015).

CHAPTER THREE

MATERIALS and METHODS

3.1 Species of bacteria

3.1.1. *Azospirillum brasilense* and *Flavobacterium* strains

Azospirillum brasilense and *Flavobacterium* strains were obtained from the Department of Biofertilization, Environmental, Natural Resources and Desertification Research Institute, National Centre for Research.

3.1.2 Isolation, multiplication and counting

The species were isolated and propagated using nutrient agar and broth media, the bacterial colonies were counted by count plate method according to Vincent's method (1970)**.** Slants and petri dishes were prepared with a media of Meat Extract Peptone broth, and the slants stricked with the strains as stock, the Petri dishes were inoculated after serial dilution from10^{-1}up to 10^{-8}. Two Petri dishes from10^{-4} and 10^{-8} were inoculated and 2 Petri dishes were left as control and kept in the incubator at 30 C° for 72 hours, observing the bacterial growth every day. After 72 hours there was uncountable growth in $10^{-4}$and a lot of growth, but countable in 10^{-8}, colony forming units (cfu)counts of bacterial growth was done from 10^{-8} and found 297 and 302 respectively and the mean was 300cfu. The Petri dishes inoculated with 10^{-4} showed uncountable growth and the high number of bacterial cfu of 10^{-8} indicated that the great competition may be the reason for suppression of bacterial growth in 10^{-4}serial dilution. The control Petri dishes were clear with no growth and the mean was 300cfu in 10^{-8}, and accordingly the calculations were done·

3.1.3 Gram staining

Bacterial cultures were prepared for gram staining by the Vincent's method (1970). For observation under light microscope, a slide of isolated and purified bacterial culture was taken and a drop of thin smear was prepared on a glass slides. The smear was air-dried; heat fixed, stained with crystal violet for one minute and slightly washed with distilled water. The smear was then flooded with iodine solution for one minute and decolorized with 95% ethanol for one minute. The smear was again washed with distilled water and counter stained with safranin. The slide was washed with distilled water, air dried and observed under light microscope (Nikon, Japan) at 100x magnification using oil immersion.

3.2 Pot experiment

Pot experiment was conducted for 12 weeks in the greenhouse facility of the College of Agricultural studies Shambat, Sudan University of Science and Technology, to study the effect of *Azospirillum brasilense* inoculation and organic fertilizer application rate on growth and growth components of sweetpotato under greenhouse conditions, using 5kg Shambat top soil (0-30cm), heat sterilized at 180c° for 2 hours by an Oven using Tyndailzation method then cooled, weighted and packed in black polyethylene plastic bags size 30 x20 cm each bag was filled by 5kg of soil. Sweetpotato clean cuttings were obtained from farmer's plots in Al Seliet Agricultural Scheme, Khartoum state, propagated in small plots before planting in the pots. *Azospirillum brasilense* inoculum strain was kindly supplied by Biofertilization Department, Natural Resources and desertification and Environment Research institute, National Centre for Research, Khartoum. The inoculum propagated in the lab. a set of biochemical tests were done including colony forming units (Cfu) counts. The three levels of *Azospirillum*

brasilense inoculum applied were (0, 10^4 and 10^8Cfu) respectively and the three rates of Al Khaseeb Organic fertilizer were (0t/ha, 25t/ha and 50t/ha), the experiment was set in factorial arrangement in a Completely Randomized Design, with three replicates. The organic fertilizer was mixed with the soil before planting and the pots were watered with adequate amount of tap water the next day clean sweetpotato apical vine cutting 25 cm in length were plant one in each pot and immediately watered after planting with suitable amount of water. *Azospirillum* inoculum was prepared in the laboratory By inoculating the broth which consist of meat extract 5g/L, sodium chloride (Na Cl) 5g/L and peptone7g/L, with the strain of *Azospirillum brasilense* and kept on rotary shaker at 150 rpm for 96 hrs for growth, and then a serial dilutions were done (10^1 to 10^8) for cfu counting. The inoculum was added from 10^4 and 10^8 according to the cfu count result. Daily observations of the plants growth were recorded. Whitefly insecticide "Decis 250"was sprayed when whitefly infestation of the plants observed. The plants were watered regularly with tap water as required and after 12 weeks the plants were harvested by separating the shoot carefully and the root from the adhered soil, washed with tap water and the shoot and root length were measured in cm then the shoot fresh and root fresh weights were determined with electric balance and placed in yellow paper envelops to dry in an Oven at 70Cº for 48 hrs. Then the dry weights were determined.

Table (3.1) The pot experiment treatments

T1	Control.
T2	*Azospirillum*10^4+0 t/ha. Of Al Khaseeb organic fertilizer.
T3	*Azospirillum*10^8+0 t/ha. Of Al Khaseeb organic fertilizer.
T4	25 t/ha of Al Khaseeb organic fertilizer.
T5	*Azospirillum*10^4+25 t/ha. Of Al Khaseeb organic fertilizer.
T6	*Azospirillum*10^8+25 t/ha. Of Al Khaseeb organic fertilizer.
T7	50 t/ha of Al Khaseeb organic fertilizer.
T8	*Azospirillum*10^4+50 t/ha. Of Al Khaseeb organic fertilizer.
T9	*Azospirillum*10^8+50 t/ha. Of Al Khaseeb organic fertilizer.

3.2.1 Soil preparation

Five kg of Shambat top soil (0-30cm), was used, heat sterilized at 180c° for 2 hours in an Oven using Tyndailzation method then cooled, weighted and packed in polyethylene plastic bags each contain 5kg soil.

3.2.2 Planting materials

The sweetpotato cultivar was obtained from farmers plots in El Seliet Agricultural Scheme, Khartoum State, named *Nigiery* and it's (orange fleshed variety) and commonly planted in the state. The cultivar was locally propagated in small plot in the farm of College of Agricultural Studies, Sudan University of Sciences and Technology in Shambat as stock for any further planting requirements. Before planting the plastic bags were opened and Al Khaseeb organic fertilizer was mixed thoroughly with the soil and watered. On next day a healthy apical cutting of 25 cm length was planted in each pot and immediately watered with tap water. After that the plants regularly watered with tap water as required. Insecticides for combating white fly and other infections were applied as required.

3.2.3 Shoot fresh weight (g)

After 12 weeks of growth the plants were harvested by cutting the shoot system above the soil surface and samples of shoot system were taken, placed in yellow paper bags and oven dried at 70 c° for 48 hours or till constant weight is gained, moisture content was determined according to the Formula:-

$$\% \text{ Moisture content} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Percentage of shoot dry weight was determined by subtracting the moisture content percent from hundred.

3.2.4 Root length (cm/ plant)

Roots of the plants are separated from the shoot at harvest, then the roots length was measured with the normal glass ruler and recorded in centimenters (cm).

3.2.5 Shoot dry weight (g)

After separating the sweetpotato shoot from the roots at harvest, the shoots were placed in yellow paper bags and Oven dried till constant weight using electric sensitive balance and then shoots dry weights were recorded .

3.2.6 Root dry weight (g)

Same as done for the shoots the roots were placed in yellow paper bags and Oven dried till the constant weight is gained using electric sensitive balance and then roots dry weights were recorded.

3.2.7 Leaf colour grading

Leaf colour grading degree is counted out of four visually as indicator for the leaf chlorophyll content.

3.3 Field experiment 1 (Sudan University) Shambat Site1

A field experiment was conducted for 6 months in the farm of the College of Agricultural studies, Sudan University of Science and Technology Shambat, to study the effect of *Azospirillum brasilense*, *Flavobacterium spp* inoculations and Al Khaseeb organic Fertilizer application rate on growth and yield of sweetpotato under field conditions. *Azospirillum brasilense* and *Flavobacterium* biofertilizers were obtained from Biofertilization Department, Environment, Natural Resources and Desertification Research Institute, National Centre for Research, Khartoum. The inoculum propagated in the lab and a set of colony forming units (Cfu) counts tests were done. The inoculum of *Azospirillum brasilense and Flavobacterium* Biofertilizers applied at the rate of (10^8Cfu/ml) with three levels of Al Khaseeb organic fertilizer (0t/ha 0.8t/ha and 1.2t/ha) applied before planting. The experiment was set in factorial arrangement in a Randomized Complete Block Design, with three replicates. Sweetpotato clean apical vine cutting 25 cm in length were planted, in the plots and immediately watered after planting. *Azospirillum* and *Flavobacterium* biofertilizers amount were determined after the (Cfu) counting and were applied at planting. Daily observations were recorded of the plants growth. Whitefly insecticide “Decis 250” was sprayed when whitefly infestation was observed on the plants. The plants were watered regularly with tap water as required and grown for 6 months. Before harvesting shoots samples were collected with plant shoot cutter and placed in yellow paper envelops to dry in hot air driven oven at 70ºC for 96 hrs, for dry weights determination. The plants were harvested by separating the shoot carefully at soil surface, total shoots fresh weights per plot were determined by weighing all the shoots using Electric balance Model AND HV-60 KGL, Japan.

Table (3.2) The field experiments treatments

T1	Control.
T2	*Azospirillum* brasilense
T3	*Flavobacterium spp.*
T4	0.8t/ha of Al Khaseeb organic fertilizer.
T5	*Azospirillum* +0.8 t/ha of Al Khaseeb organic fertilizer.
T6	*Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer.
T7	*Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer.
T8	1.2 t/ha of Al Khaseeb organic fertilizer.
T9	*Azospirillum*+1.2t/ha of Al Khaseeb organic fertilizer.
T10	*Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer.
T11	*Azospirillum*+ *Flavobacterium* +1.2 t/ha of Al Khaseeb organic fertilizer.

3.3.1 Soil preparation

Land preparation included one round of disc ploughing followed by one round of disk harrowing and ridges at 60 cm apart are then built using a tractor-mounted ridger. The size of ridge is 30 cm high and 60 cm width at the base after land preparation the organic fertilizer Al Khaseeb was applied according to the treatments and watered.

3.3.2 Planting sweetpotato

Healthy clean sweetpotato cuttings vine 25 cm in length was prepared from locally propagated sweetpotato using the pruning cutter; the cuttings were planted in 30 cm distance and immediately watered after planting. As indicators for the sweetpotato growth stem length (cm), number of branches per plant and number of leaf per plant were determined during the growth period at 6 and 16 weeks after planting (WAP).

3.3.3 Data collection

3.3.3.1 Stem length (cm)

Randomly four plants per plot were selected and stem length was measured using plastic type meter and the sum is averaged by dividing by four, to represent a replicate reading.

3.3.3.2 Number of branches /plant

Number of branches per plant were counted from randomly selected four plants per plot and then averaged to represent a replicate, and then the three replicates were averaged again to obtain the treatment mean.

3.3.3.3 Leaf number/ plant

Number of leaf per plant were counted from randomly selected four plants per plot and then averaged to represent a replicate, and the three replicates then averaged again to obtain the mean for the leaf number per plant for each treatment.

3.3.3.4 Leaf chlorophyll content

Leaf chlorophyll content SPAD 502 values were measured six weeks after planting using SPAD502 meter, taking three sample readings then averaged to represent a plot mean.

3.3.3.5 Shoot dry matter percentage

Before harvesting the sweetpotato samples of shoots for dry weight determination and nutrient content analysis were collected from the middle rows of each plot and placed in yellow paper 15 x 10 inch envelopes and dried till constant weight was recorded.

3.3.3.6 Shoot total fresh weight

At the time of harvest the total shoots (Leaf and stems) of sweetpotato of every plot were collected together and weight to determine the shoots fresh weight of sweetpotato per plot and the three readings were averaged to get the treatment mean .

3.3.3.7 Storage roots yield

Immediately after harvesting the sweetpotato storage roots fresh weights were recorded per plot in (kg) using Electric balance Model AND HV-60 KGL, Japan.

3.3.3.8 Storage roots dry matter percentage

Four storage roots of different size were selected per treatment, washed with tap water left on bench to dry for a while then divided with kitchen knife to three parts and the meddle portion of the storage roots were sliced, the fresh weight was recorded and placed in yellow paper bags and oven dried at 70 c° for 48 hours or till constant weight is gained, then the storage roots dry weights were recorded and storage roots moisture percentage was calculated by subtracting the dry weight percentage from hundred.

3.3.3.9 Sample storage roots yield (kg/plot)

At the time of harvest the yield of storage roots from the meddle two rows of each plot was weighed with electric balance in Kg to represent a sample yield per plot, however all the four rows yield of storage roots was recorded as total yield per plot and weight in kg and the three replicates were averaged to represent the treatment mean, and duly the storage roots yield in t/ ha was calculated.

3.3.3.10 Storage roots yield (t/ha).

According to the storage roots yield per plot determined previously and taking in account the dimensions of the plot and the storage roots yields in kilograms the storage roots yield in ton per hectare was calculated.

3.3.3.11 Marketable Storage roots yield (kg/ plot)

The storage roots yield was classified or graded to marketable storage roots and none marketable storage roots according to the roots characteristics, volume, weight and infestation percentage and the small storage roots and highly infested big storage roots were graded as non-marketable and the weight of marketable storage roots per plot was recorded, and then the three replicates averaged to represent the treatment mean of marketable storage roots weight in (kg).

3.3.3.12 Non-marketable storage roots yield

After the storage roots harvest, the yield was classified to marketable storage roots and non-marketable storage roots according to the storage roots characteristics and the non-marketable storage roots weight per plot was determined, and then the three replicates were averaged to represent the treatment mean of non-marketable storage roots weight in (kg).

3.3.3.13 Marketable storage roots yield (t/ha)

After the sorting or grading the storage roots yield to marketable storage roots and non-marketable storage roots and marketable storage roots were weighed in kg per plot and accordingly the marketable storage roots yield in t/ ha was calculated.

3.3.3.14 Non-marketable storage roots yield (t/ha)

After determining the non-marketable storage roots yield weight, the non-marketable storage roots were weighed in kg per plot and accordingly the non-marketable storage roots yield in t/ha, per treatment was calculated.

3.3.3.15 Shoots total (N %), Protein and organic carbon content

Total nitrogen of sweetpotato shoots was determined by the Kjeldahl method with a representative 2g powder, and then total nitrogen was multiplied by factor 6.25 to obtain protein content of sweetpotato shoots (AOAC, 1990).

3.3.3.16 Storage roots specific gravity

Four storage roots of different sizes and volumes were selected randomly, washed with tap water to remove any adhering soils and to add moisture to prevents the storage roots skin dryness, then weighed with electric balance in grams and a big size baker was filled with tap water, the storage root placed in the baker and the volume of storage roots were determined by measuring the displaced water with 1000 ml graded cylinder. Then by dividing the mass or weight of storage roots by the determined volume, the specific gravity was calculated.

3.4 Field Experiment 2 (University of Gadarif) Tawawa Site2

A field experiment was conducted for 6 months in the experimental farm of the Faculty of Agricultural and Environmental Sciences, University of Gadarif, Tawawa, as second site and the Materials used and Methods followed were as mentioned in section 3.3 and sub sections in field experiment1.

3.5 Statistical analysis of the Data

Data were analyzed using SPSS14.0 computer Program. The analysis of variance (ANOVA) was done for treatment means; also treatments means separation was done using Least Significant Difference (LSD) and Duncan's Multiple Range Test (D M R T) (Gomez and Gomez, 1984).

CHAPTER FOUR

RESULTS and DISCUSSION

The pot experiment soil was Shambat top soil 0-30 cm and the soil chemical and physical properties are mentioned in table (A) in Appendix.

The field experiments were conducted in tow sites; experimental Farm of College of Agricultural Studies (CAS), Sudan University of science and Technology "Shambat" site 1 and the experimental Farm, Faculty of Agricultural and Environmental Sciences, University of Gadarif "Tawawa" site 2, both sites soils chemical and physical properties are mentioned in table (B) and table (C) in the Appendix.

4.1 Pot experiment

Effect of organic Fertilizer application rate and *Azospirillum brasilense* inoculum intensity on growth and growth components of sweetpotato (*Ipomoea batatas* L).

4.1.1 Data collection

4.1.2 Shoot fresh weight (g)

The obtained results showed an increase in sweetpotato shoot fresh weight with application of (T7) 50 t/ha of Al Khaseeb organic fertilizer alone, indicating the great influence of organic fertilizer on shoot growth, followed by application of (T3) *Azospirillum Brasilense* with 10^8, and these shows the potential of *Azospirillum Brasilense* biofertilizer in improving plant growth, reducing the production cost and conserving the natural resources. Meanwhile

the application of (T5)*Azospirillum* biofertilizer 10^4 + 25 t/ha of Al Khaseeb organic fertilizer showed the third most high effect indicating to the importance of co-inoculation of both the *Azospirillum Brasilense* biofertilizer and Al Khaseeb organic fertilizer in sweetpotato growth improvements compared to (T1) the uninoculated control Figure(4.1) Similar results were obtained by Singh, *et al.*, (2010),who reported" that *Azospirillum Brasilense* can improve the growth and yield of several plants including economically important cereals and grasses".

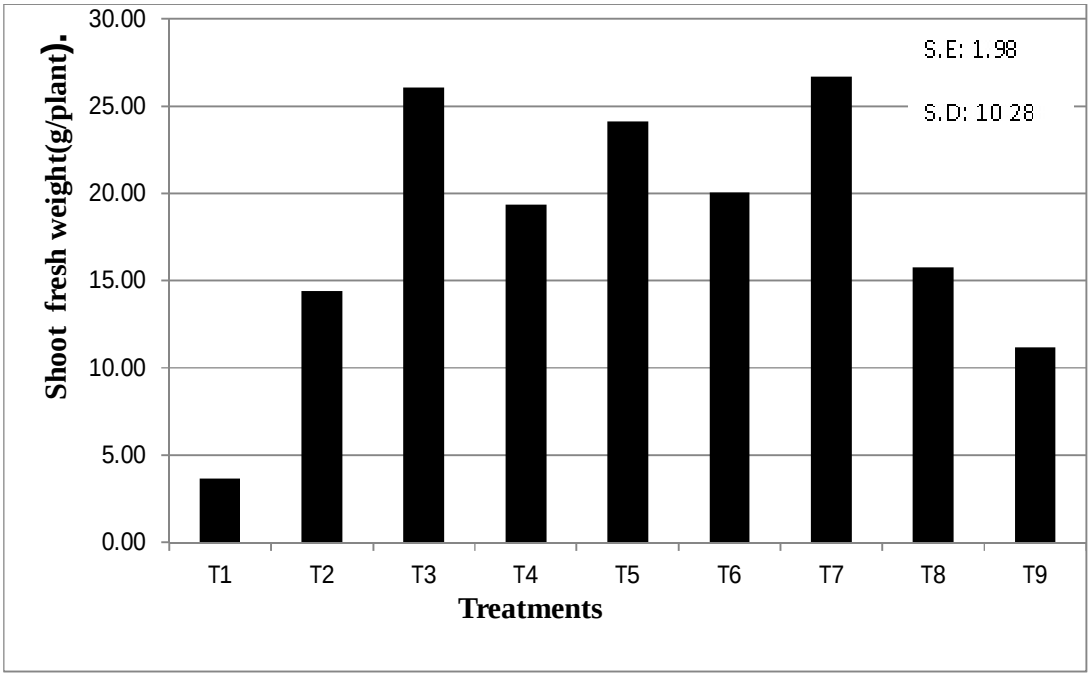

Figure: (4.1) Effect of *Azospirillum brasilense* and AI Khaseeb organic fertilizer on shoot fresh weight (g/plant).

4.1.3 Root length (cm/plant)

The obtained results revealed that inoculation of sweetpotato with (T5) *Azospirillum brasilense* 10^4cfu +25 t/ha of Al Khaseeb organic fertilizer, showed

almost similar effect to the application of 50t/ha of (Al Khaseeb) organic fertilizer alone on root length, indicating that with *Azospirillum* biofertilizer inoculation, 50% of the organic fertilizer need could be saved. Meanwhile inoculation with (T6) *Azospirillum brasilense*10^8 cfu+25t/ha of organic fertilizer showed an increase in root length followed by inoculation with (T2) *Azospirillum brasilense* 10^4cfu+ 0 t/ha of Al Khaseeb organic fertilizer and inoculation with (T3) *Azospirillum brasilense* 10^8 cfu alone compared to the control figure (4.2) this results are in line with the findings of Farzana, *et al*., (2007)who reported that "the inoculation process enhanced plant growth which could be related to enhancement of root growth and higher nutrient uptake" .

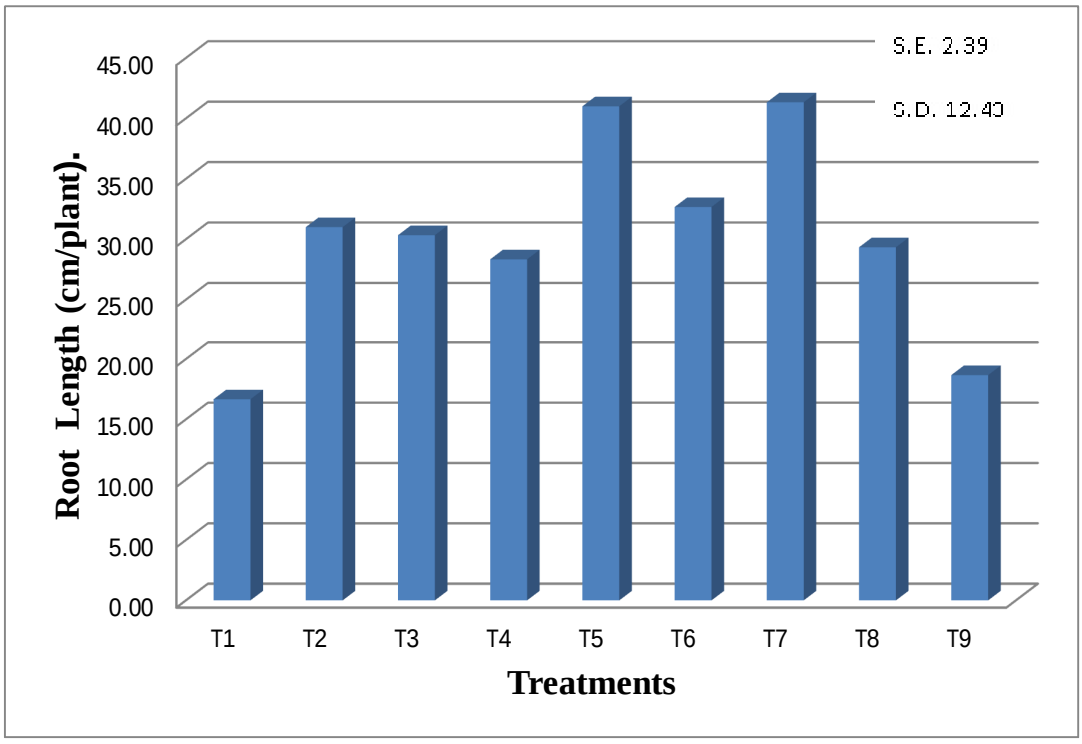

Figure :(4. 2) Effect of *Azospirillum brasilense* and AI Khaseeb organic fertilizer on root length (cm/plant).

4.1.4 Shoot dry weight (g/plant)

Obtained results showed that inoculation with(T3) *Azospirillum* Spp. 10^8 cfu+ 0 t/ha of Al Khaseeb organic fertilizer effect was the most high on

sweetpotato shoot dry weight followed by inoculation with(T5) *Azospirillum brasilense* 10^4 cfu+ 25 t/ha of Al Khaseeb organic fertilizer and the third most high effect was observed in application of (T7) 50 t/ha of organic fertilizer alone, Followed by inoculation with (T6) *Azospirillum brasilense* 10^8 cfu+ 25 t/ha of Al Khaseeb Organic fertilizer and application of (T4) 25 t/ha of Al Khaseeb organic fertilizer alone compared to un inoculated control figure(4.3). These results are in line with the findings of the Bashan, *et al,*. (1990) who reported that "All *Azospirillum brasilense* strains significantly at ($P < 0.05$) improved wheat and soybean growth by increasing root and shoot dry weight and root surface area".

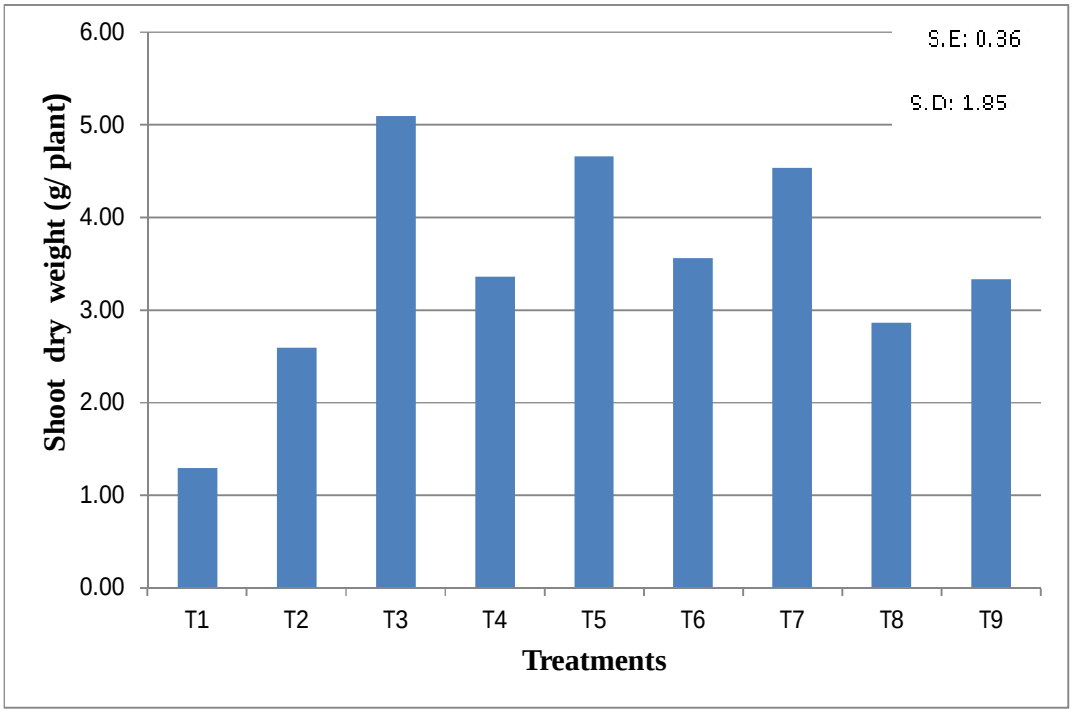

Figure :(4.3) Effect of *Azospirillum brasilense* and AI Khaseeb organic fertilizer on shoot dry weight (g/plant).

4.1.5 Root dry weight (g/plant)

The results revealed that application of (T7) 50 t/ha of Al Khaseeb organic fertilizer alone showed the highest effect on sweetpotato root dry weight.

Followed by inoculation with(T8) *Azospirillum brasilense* 10^4 +25 t/ha of Al Khaseeb organic fertilizer, however inoculation with(T6) *Azospirillum brasilense* 10^8 +25 t/ha of Al Khaseeb organic fertilizer showed less effect than (T8)this may be due to high competition. These can indicate the essential role of the suitable dose of organic fertilizer in activation of *Azospirillum brasilense* biofertilizer. On the other hand (T2) *Azospirillum brasilense* 10^4cfu+ 0 t/ha of Al Khaseeb organic fertilizer and (T9) *Azospirillum*+1.2t/ha of Al Khaseeb organic fertilizer, showed almost similar effect on root dry weight. All treatments showed an increase in root dry weight value compared to control without inoculation and without fertilization Figure (4.4) similar results were obtained by(Farzana, *et al*.,2007).

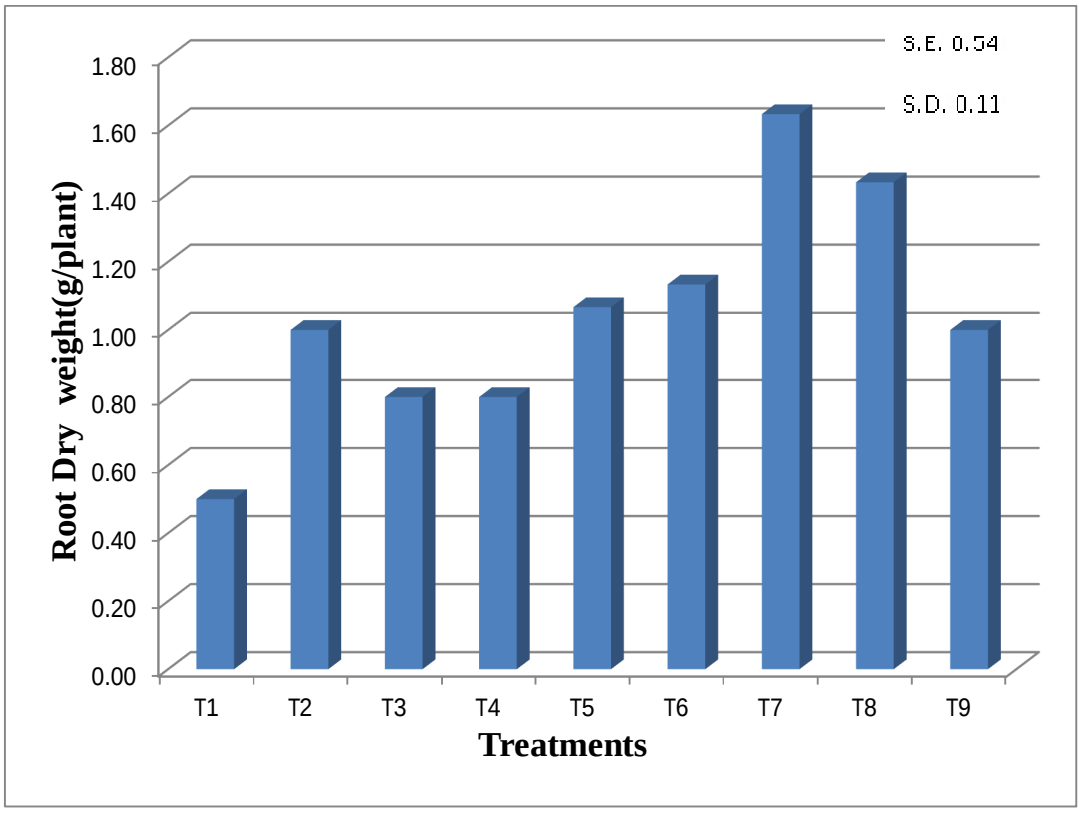

Figure (4.4): Effect of *Azospirillum brasilense* and AI Khaseeb organic fertilizer on root dry weight (g/plant).

4.1.6 Colour rating

The color rating results showed that a combination of inoculation with (T5) *Azospirillum brasilense* 10^4 cfu+ 25 t/ha of Al Khaseeb organic fertilizer gave the highest effect with more than 3.5 out of 4,indicating to the most suitable application rate of Al Khaseeb organic fertilizer and inoculation intensity of *Azospirillum brasilense*, followed by treatments (T2, T3, T7 and T8) with 3 out of 4 in colour rating degrees, Compared to the control without inoculation and without fertilization figure (4.5), color rating is indicator for plants nutrition status and Leaf chlorophyll content and leaf chlorophyll content is indicator for good and high yield of the crop, similar results were reported by Kowsar, (2014).

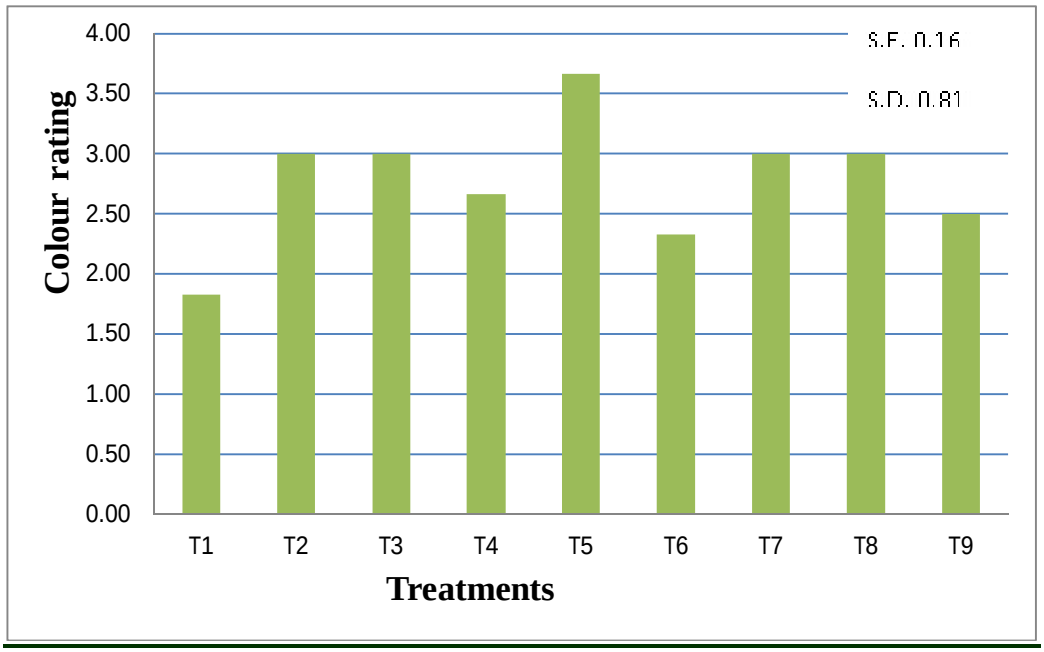

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Figure:(4.5) Effect of *Azospirillum brasilense* and AI khaseeb organic fertilizer on leaf colour grading.

4.2 Field experiment Shambat (CAS) Sudan University

4.2.1 Field experiment 1

A. Sweetpotato growth components at six weeks after planting (WAP).

4.2.2 Stem length

Regarding the sweetpotato stem length after (6WAP) results showed that application of (T9) organic fertilizer 1.2 t/ha + *Azospirillum*) showed the highest effect on sweetpotato stem length followed by application of (T7) organic fertilizer 0.8 t/ha + *Azospirillum*+ *Flavobacterium* and application of (T2) *Azospirillum* alone, indicating the great potential of *Azospirillum* biofertilizer in improving the stem length proliferation and sweetpotato plant growth as general. Meanwhile (T8) organic fertilizer 1.2 t/ha alone showed the least value in stem length in Shambat site. However (T1) the control showed almost similar value with (T3) *Flavobacterium spp.* and T6): *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer Figure (4.6) similar results were obtained by (Martinez-Toledo, *et al.*, 1988).

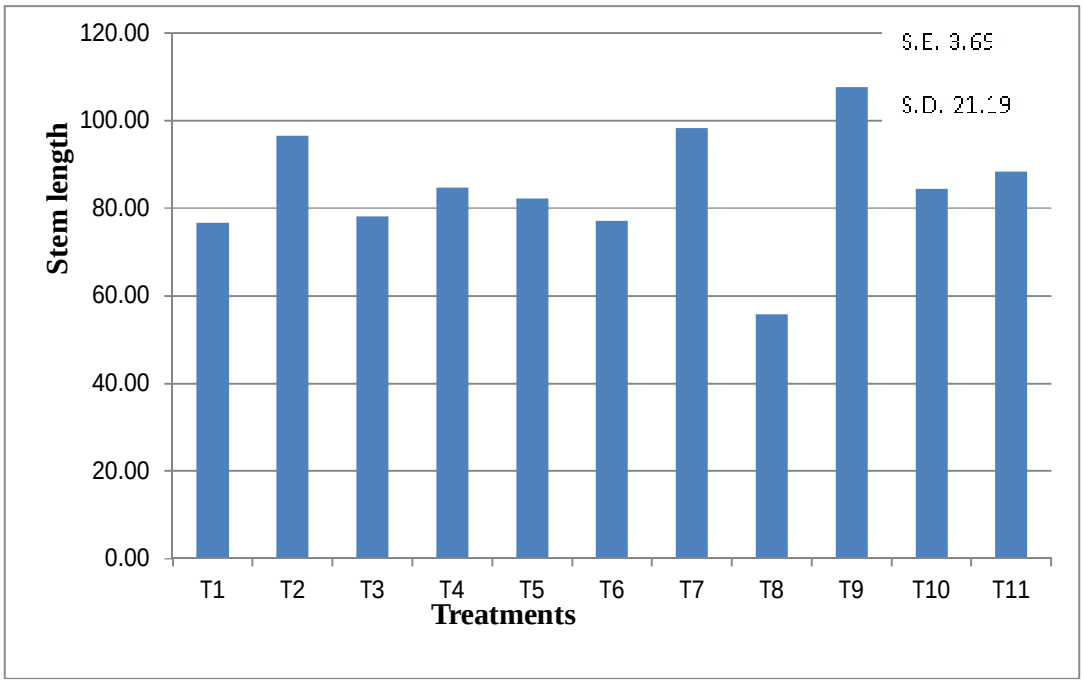

Figure (4.6). Effect of biofertilization on stem length (cm/plant) at six weeks after planting (6WAP).

4.2.3 Leaf number

In number of sweetpotato leaf after (6WAP) results revealed that application of (T9) organic fertilizer 1.2 t/ha + *Azospirillum*) showed statistically significant ($P < 0.05$) differences effect on sweetpotato leaf number followed by application of (T7) organic fertilizer 0.8 t/ha + *Azospirillum*+ *Flavobacterium*, and it's clearly observed that (T8) organic fertilizer 1.2 t/ha alone also showed the least value in leaf number. However the control showed higher value than the rest of the treatments figure (4.7) in line with the current results, Chela, *et al.*, (1993) reported significantly higher plant growth due to the use of nitrogen in combination with PGPR than the Fertilization alone under field conditions increase in stem number per plant of potato in response to fertilization.

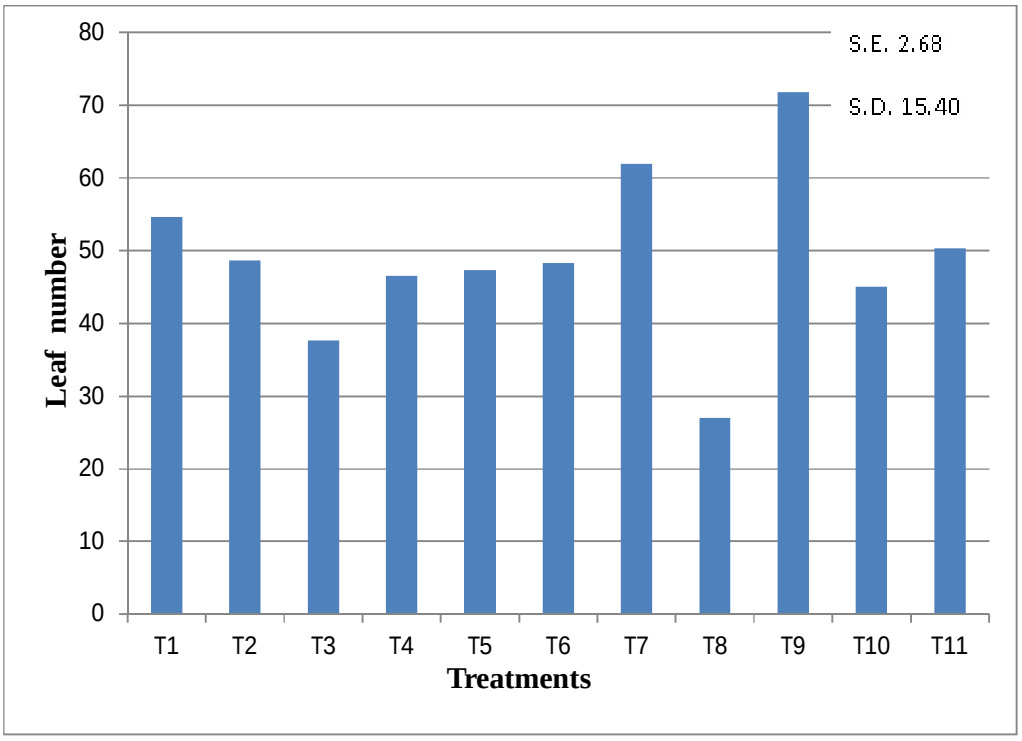

Figure (4.7). Effect of biofertilization on leaf number at six weeks after planting (6WAP).

4.2.4 Branches number

The obtained results revealed that sweetpotato plants' braches number and in site (1) Shambat; showed the same trend with leaf number and statistically significant ($P < 0.05$) differences for application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, were observed followed by application of(T7) organic fertilizer 0.8t/ha+ *Azospirillum*+ *Flavobacterium*, and it's clearly observed that (T8) organic fertilizer 1.2 t/ha alone also showed the least value in branches number. However the control showed higher value than the rest of the treatments figure (4.8), number of branch per plant is one of the most important yield components of root and tuber crop in general and sweetpotato in particular, in contrast with the current findings, Zelalem, *et al.* (2009) and Mukhtar, *et al.* (2010) found non-significant increase in stem number per plant of potato in response to fertilization..

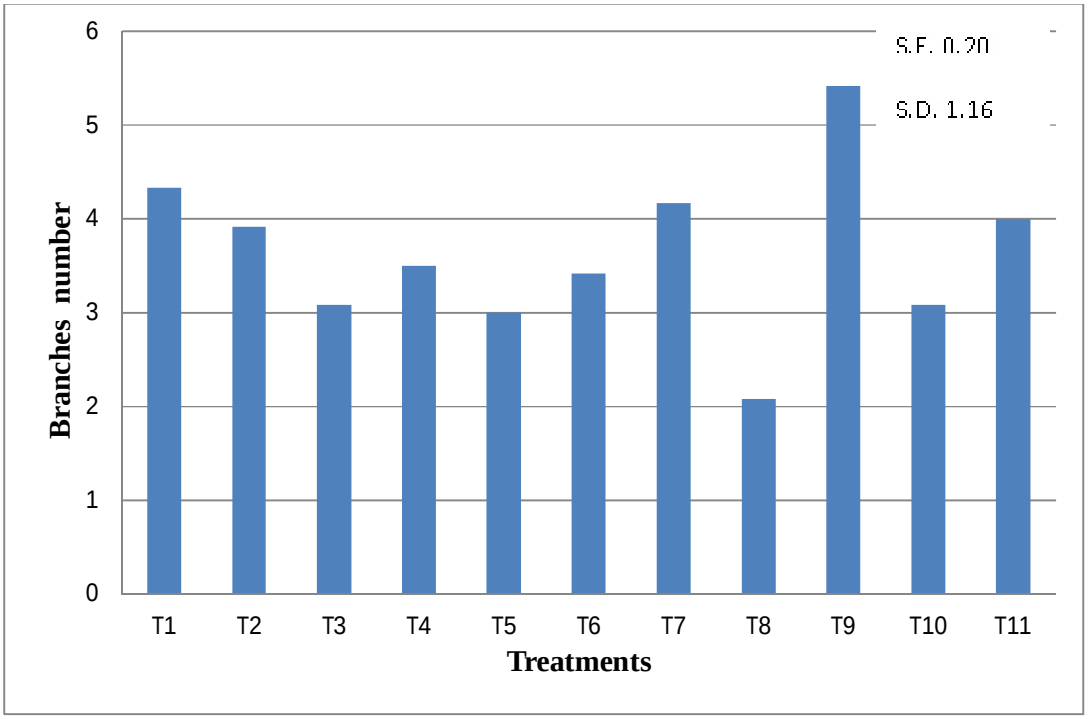

Figure (4.8). Effect of biofertilization on branches number at six weeks after planting (6 WAP).

4.2.5 Leaf chlorophyll content

Results showed that the application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum* showed great effect on sweetpotato plants leaf chlorophyll content followed by application of (T4) organic fertilizer 0.8 t/ha and application of (T10) organic fertilizer 1.2 t/ha + *Flavobacterium*. However application of (T1) the control treatment showed high value in leaf chlorophyll content is indicator for good plant nutrition and high yield, figure (4.9) similar results were obtained by Kowsar,(2014) who reported that" increased amount of chlorophyll content in leaves indicates the photosynthetic efficiency, thus it can be used as one of the criteria for quantifying photosynthetic rate" and Yoshida (1972) stated that "higher chlorophyll is one of the most important factors for better yield".

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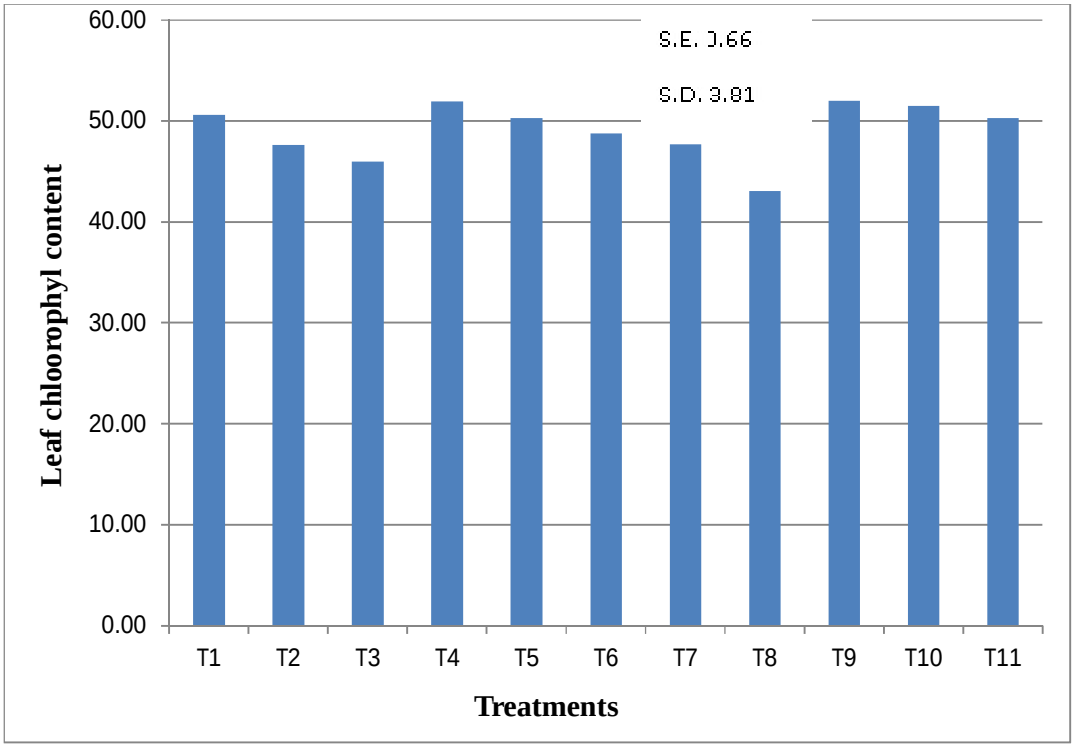
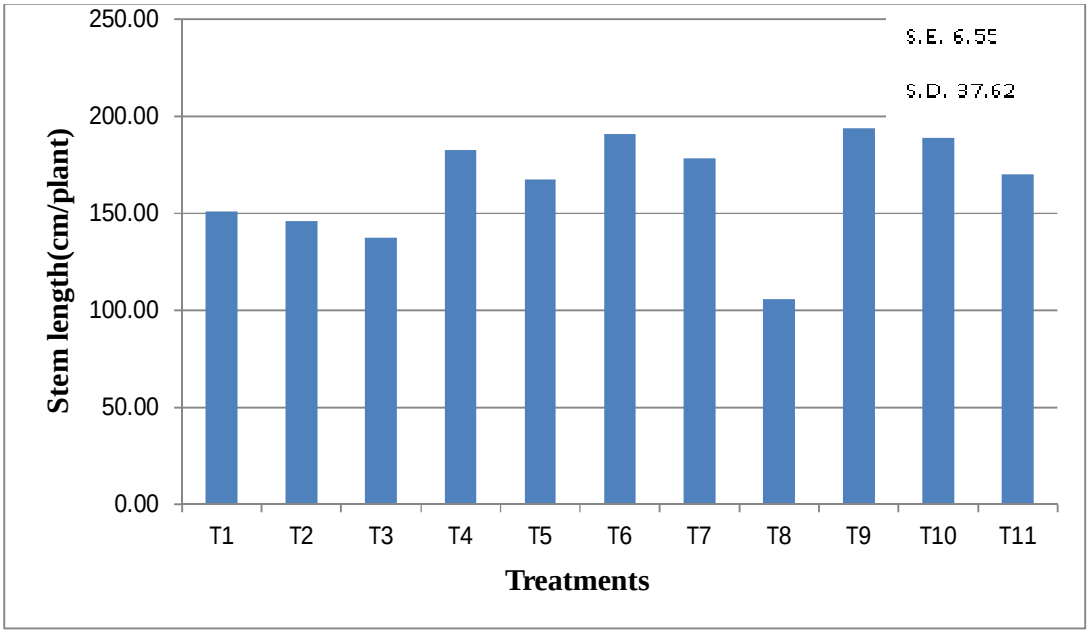


Figure (4.9). Effect of biofertilization on leaf chlorophyll content, at six weeks after planting(6WAP).

B. Growth components at sixteen weeks after planting (WAP).

4.2.6 Stem length

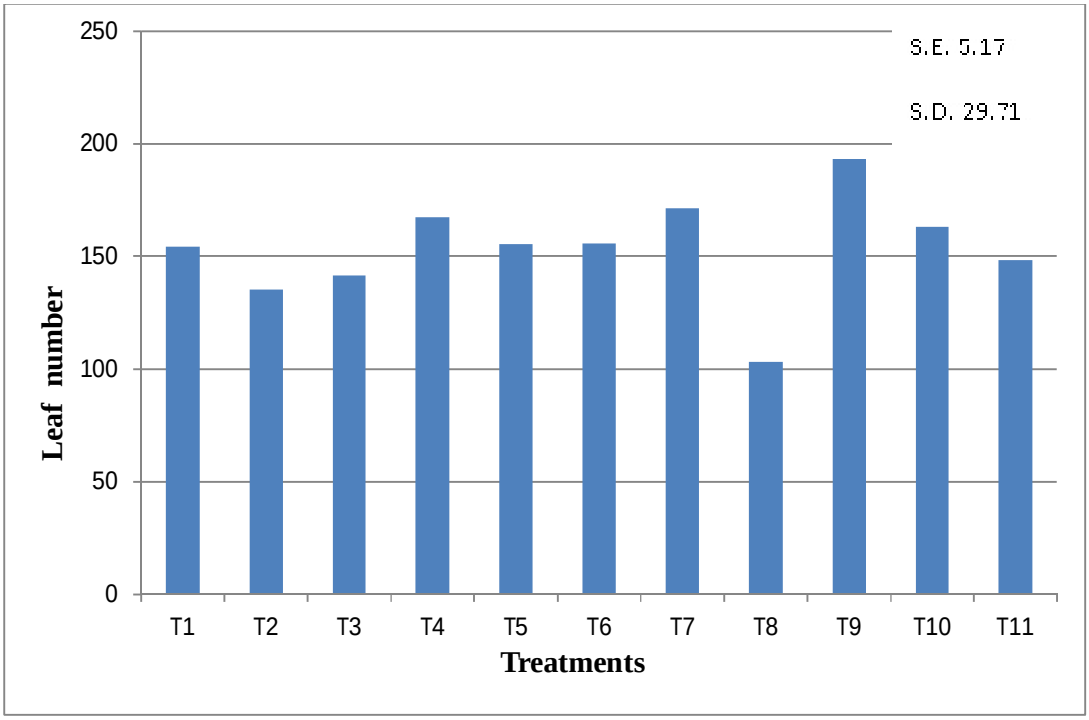
The results showed that the application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum* had the most higher effect on sweetpotato plants stem length followed by application of (T10) organic fertilizer 1.2 t/ha + *Flavobacterium* and application of (T6) organic fertilizer 0.8 t/ha + *Flavobacterium.* However the application of (T8) organic fertilizer 1.2 t/ha alone showed the lowest value in stem length after 16 (WAP) figure (4.10) similar results were obtained by (Bashan, *et al.*, 2004) when they reported that" inoculation of plants with *AzospiriIIum* resulted in significant changes in various growth parameters, such as increase in pIant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of Cereals".



Figure(4.10). Effect of biofertilization on stem length (cm/plant) at sixteen weeks after planting (16WAP).

4.2.7 Leaf number

Regarding the leaf number per plant in Shambat site after 16 (WAP) the results indicated that application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum* on sweetpotato plants leaf number showed significant effect, followed by application of (T7) organic fertilizer 0.8 t/ha + *Azospirillum* + *Flavobacterium* and application of (T4) organic fertilizer 0.8t/ha. Mean-whiles the application of (T8) organic fertilizer 1.2 t/ha had the same trend in showing the lowest value figure (4.11). Leaf number showed a considerable response to inoculation with *Azospirillum* and organic fertilizer application similar results were reported by (Bashan, *et al.*, 2004).



Figure(4.11). Effect of biofertilization on leaf number at sixteen weeks after planting (16 WAP).

4.2.8 Branches Number

The obtained results revealed that the branches number per plant in Shambat site experiment after 16 weeks after planting (WAP) showed that application of (T9) organic fertilizer1.2 t/ha+ *Azospirillum* on sweetpotato plants branches number significant effect, followed by application of (T7) organic fertilizer 0.8 t/ha + *Azospirillum*+ *Flavobacterium* and application of (T6) organic fertilizer 0.8 t/ha + *Flavobacterium*. However application of (T11) organic fertilizer 1.2 t/ha + *Azospirillum* + *Flavobacterium*, Figure (4.12)this results are in line with findings of (Bashan, *et al.*, 2004) who reported that" inoculation of plants with *AzospiriIIum* resulted in significant changes in plant biomass, plant height, Leaf size and root length. "

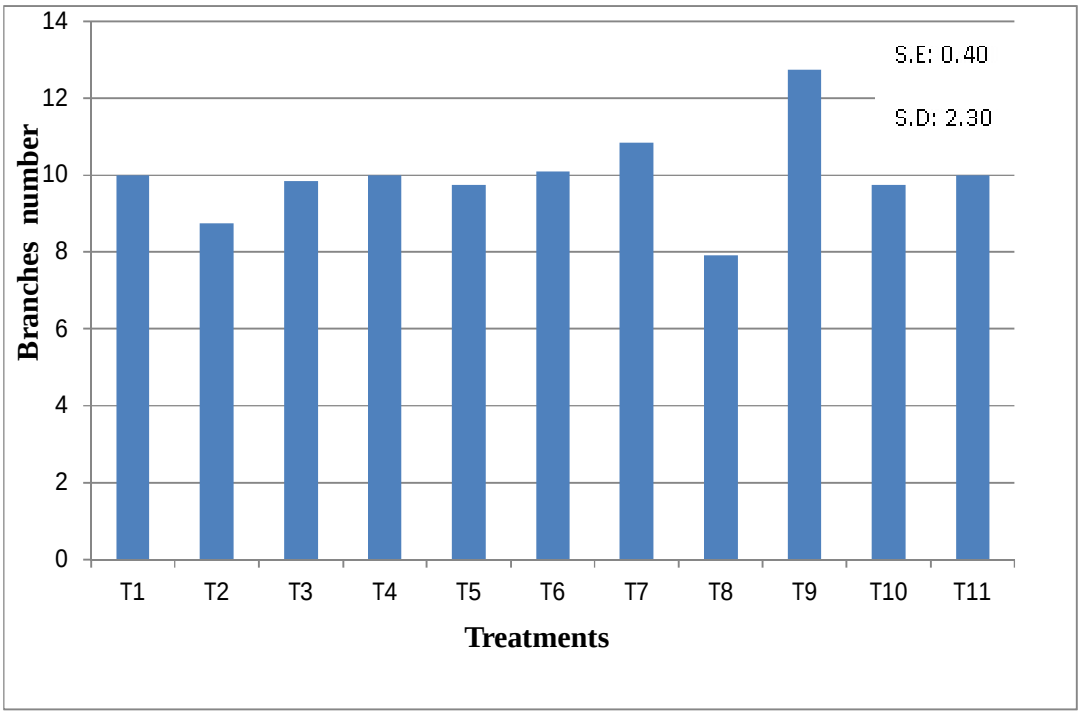


Figure (4.12). Effect of biofertilization on branches number at sixteen weeks after planting (16 WAP).

C. Harvesting data for Shambat field experiment.

4.2.9 Total shoots fresh weight (kg/plot)

Obtained results showed that no significant effect on total sweetpotato plant shoots weight and the application of (T1) the control showed the highest effect, followed by application of (T8) organic fertilizer 1.2 t/ha. However the lowest values for total shoot fresh weight was observed with application of (T4) 0.8t/ha of Al Khaseeb organic fertilizer alone and application of (T5) organic fertilizer 0.8 t/ha + *Azospirillum* figure (4.13) opposed to the current findings, Powon, *et al.*, (2005) found total fresh biomass responded positively to the combined application of Farmyard Manure (FYM) and P to potato in Kenya.

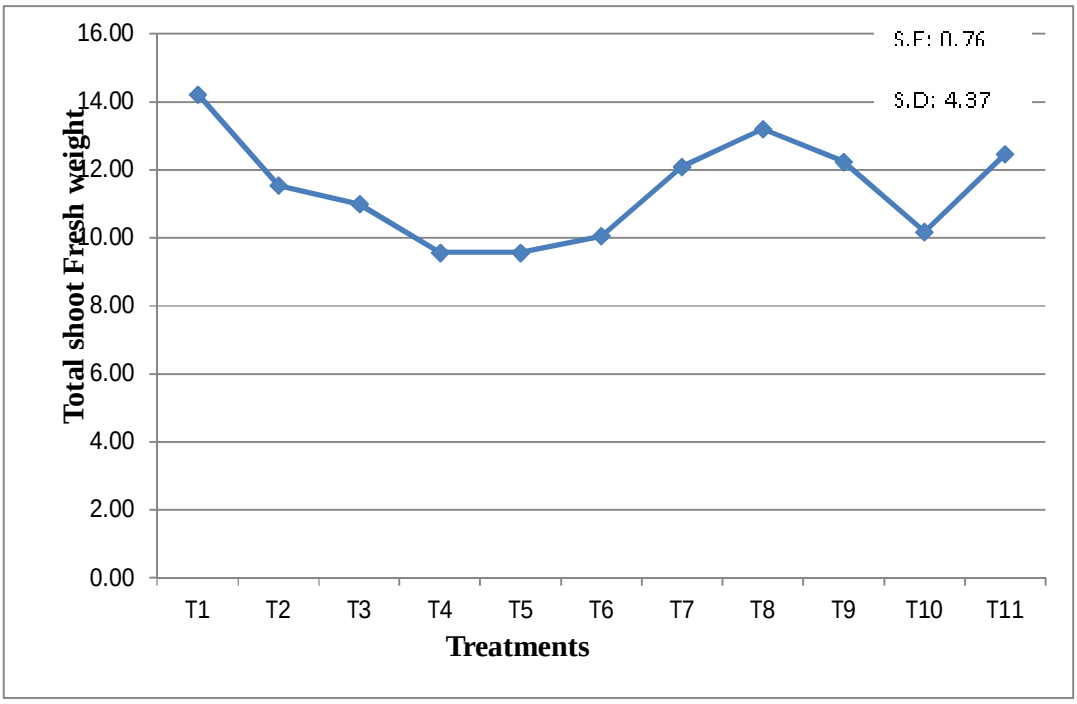


Figure (4.13). Effect of biofertilization on total shoot fresh weight (kg/plot).

4.2.10 Sample storage roots yield (kg)

Results of sample sweetpotato storage roots yield fresh weight, showed that application of (T9)organic fertilizer 1.2 t/ha+ *Azospirillum* and application of (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer showed the highest storage roots yield followed by the application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer. Meanwhile the lowest yield of storage roots was obtained with application of (T3) *Flavobacterium* spp alone Figure (4.14) similar results were obtained by Farzana, *et al*., (2007) when she reported that "in general PGPR inoculation improved the storage roots weight compared to the non- inoculated control".

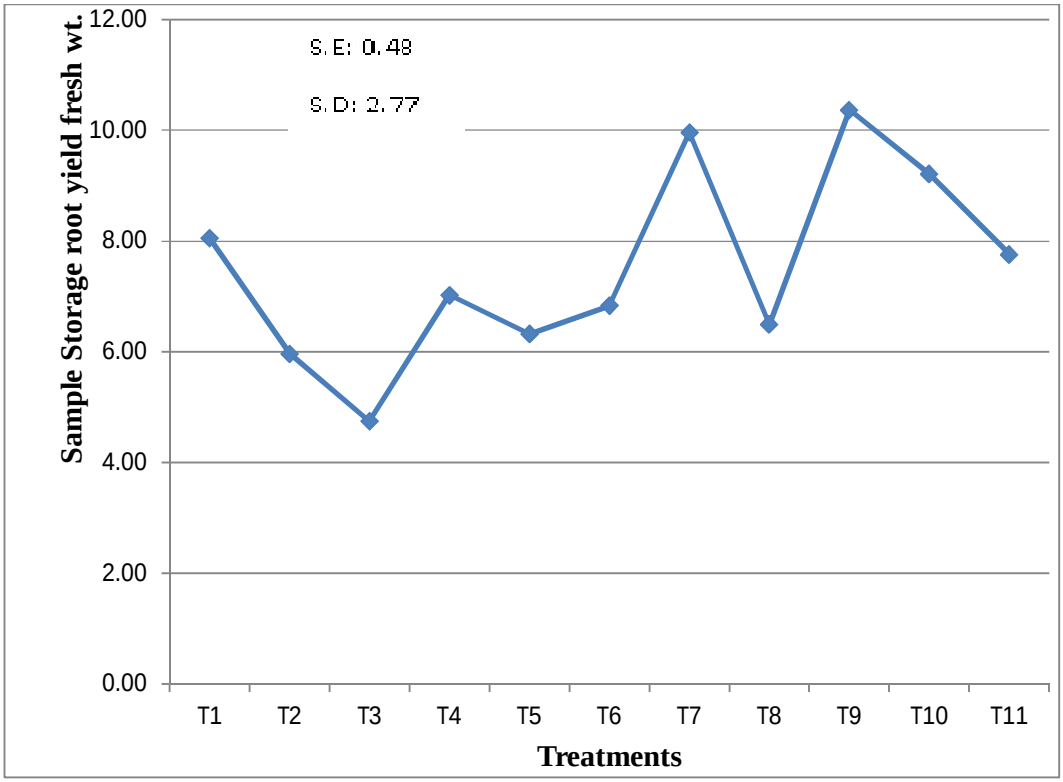


Figure (4.14). Effect of biofertilization on sample storage roots yield fresh weight (kg/pIot)·

4.2.11 Total storage roots yield (kg/plot)

The obtained results showed that the highest yield of sweetpotato storage roots fresh weight was obtained by application of (T11) organic fertilizer 1.2 t/ha + *Azospirillum* + *Flavobacterium*, followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, and the third one with application of (T1) the control. The yield of control may be due the history of pervious fertilization in site. However the least value for total yield was obtained with application of (T2) *Azospirillum* brasilense figure (4.15) this results are in line with findings of Saad, *et al.*, (1999) who found that, inoculated sweetpotato with PGPR + 1/3N produced higher root yield and plant growth of sweetpotato plants than non- inoculated plants given normal rate of N fertilizer

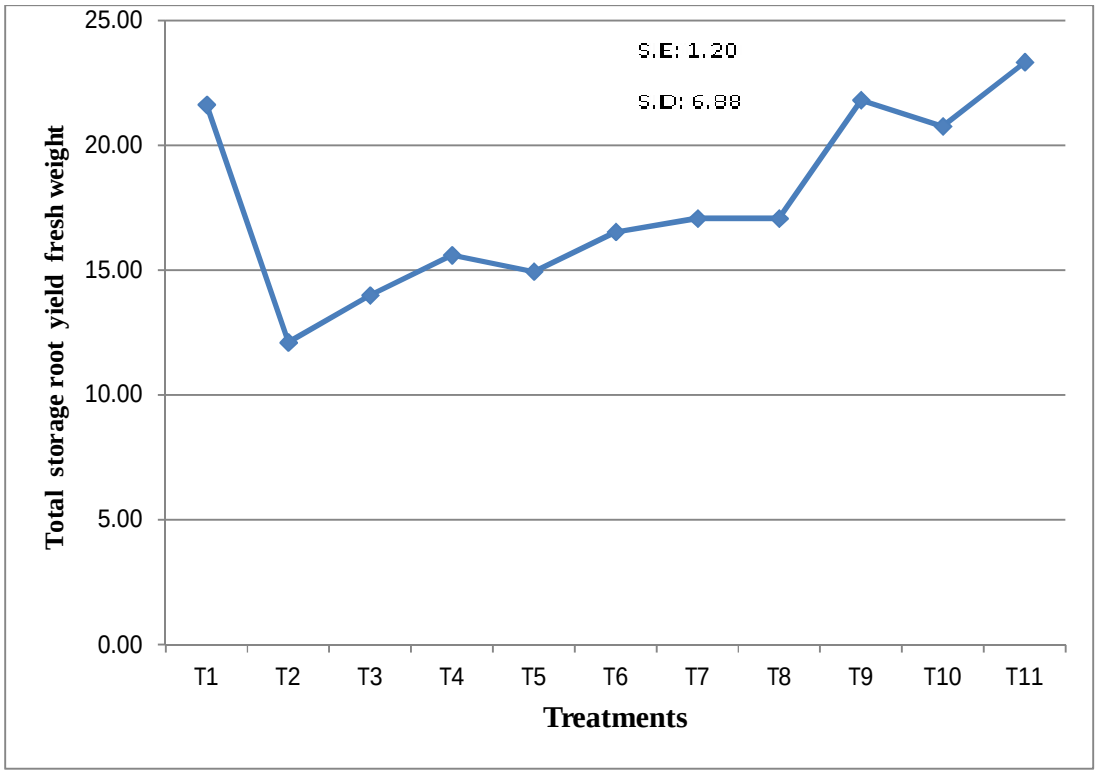


Figure (4.15). Effect of biofertilization on total storage roots yield (kg / plot).

4.2.12 Storage roots dry matter (%)

Obtained results showed the highest effect on sweetpotato storage roots dry matter percentage with application of (T8) organic fertilizer 1.2 t/ha, followed by application of (T3) *Flavobacterium* spp and application of (T5) *Azospirillum brasilense* +0.8 t/ha of Al Khaseeb organic fertilizer, but there was no significant among treatments means, generally most of the treatments showed good dry matter content. However the lowest values were observed with application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum* and application of (T4) 0.8t/ha of Al Khaseeb organic fertilizer alone figure (4.16) similar results were obtained by Teshome, (2012) who found that, Tuberous root dry biomass was not significantly ($P < 0.05$) affected by the combined application of farmyard manure and phosphorus.

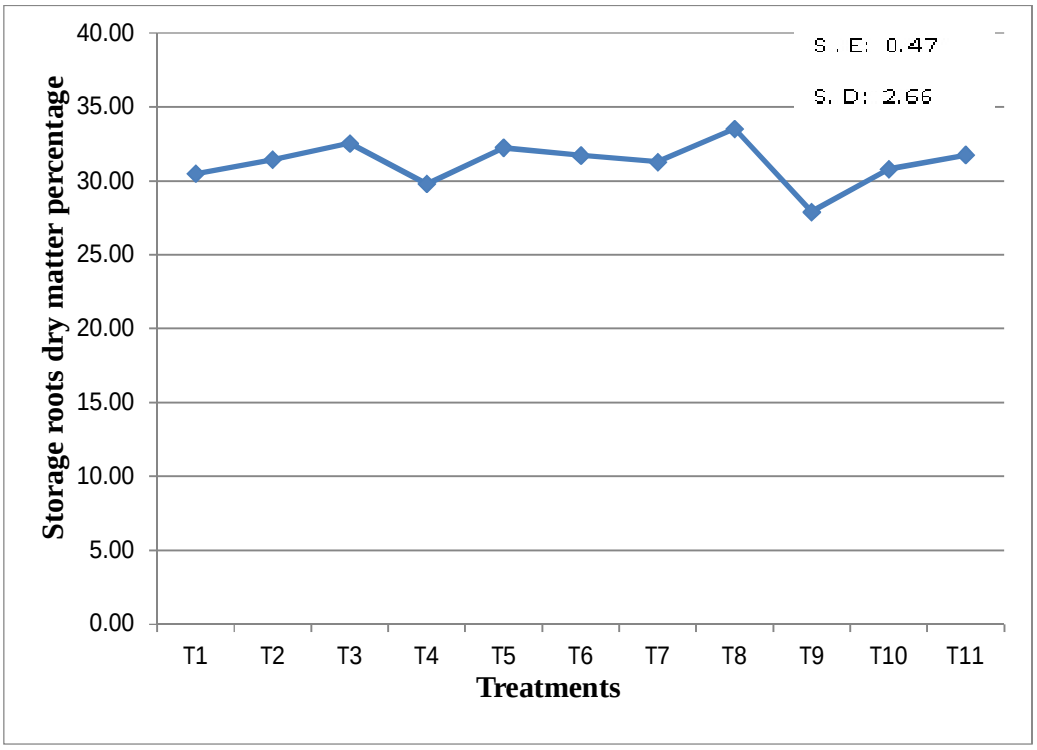


Figure (4.16). Effect of biofertilization on storage roots dry matter percentage.

4.2.13 Storage roots yield (t/ha)

The obtained results showed that sweetpotato storage roots yield in tone per hectare was greatly affected by application of (T11) organic fertilizer 1.2 t/ha + *Azospirillum* + *Flavobacterium*, followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, and the third one was with application of (T1) the uninoculated control. However the Lowest storage roots yield value was recorded with application of (T2) *Azospirillum* alone figure (4.17). This can indicate the benefits of combined inoculation of beneficial microbes in improving the sweetpotato crop yield in line with natural resources conservation and in affordable production cost, similar results were obtained by Fatima, *et al*.,(2008)who found that Use of combined treatment of N-fixers and *B. circulans* gave better plant height, stem diameter, number of branches per plant, as well as fresh and dry weight of marjoram than those obtained from either bio-fertilizer alone during three cutting.

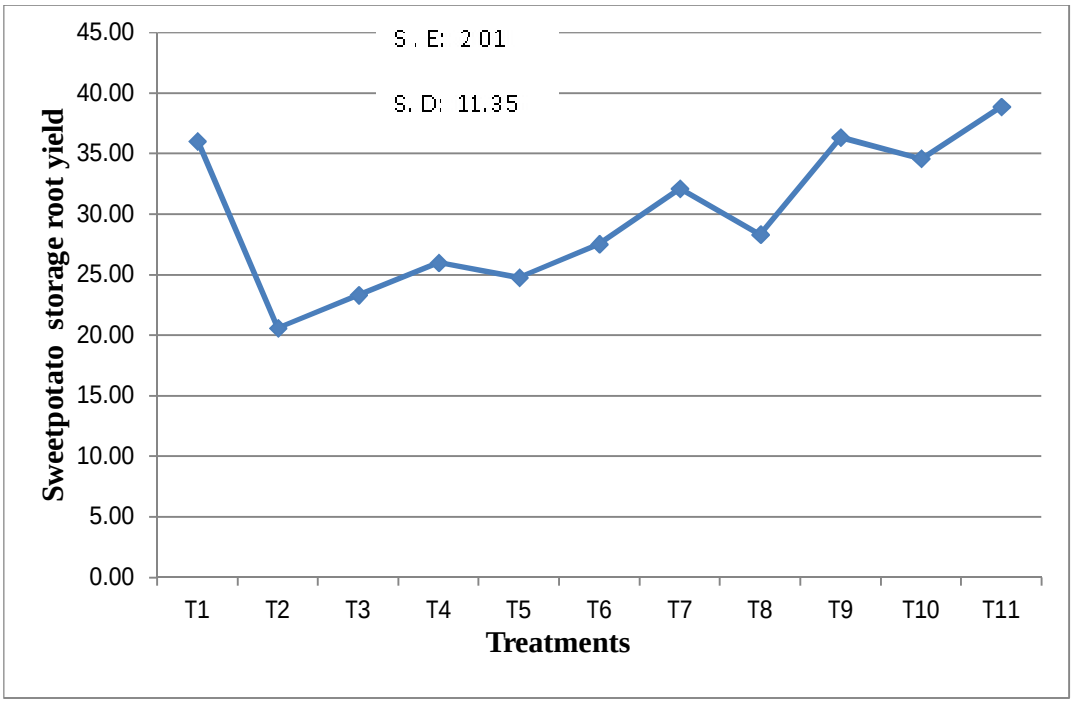


Figure (4.17). Effect of biofertilization on storage roots yield (t/ha).

4.2.14 **Marketable storage roots yield**

Regarding the quality of storage roots yield and marketable storage roots yield, the obtained results showed that application of (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of Al Khaseeb organic fertilizer had significant effect on marketable storage roots yield followed by application of (T9) Al Khaseeb organic fertilizer 1.2 t/ha + *Azospirillum*, application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer and *Flavobacterium* + 0.8 t/ha of Al Khaseeb organic fertilizer figure (4.18) indicating to the importance of organic fertilizer application and biofertilizer co-inoculation. These results are in inline with findings of Teshome, (2012), who reported the optimum marketable tuber roots yield was obtained with combined application of Farmyard manure and Phosphorus.

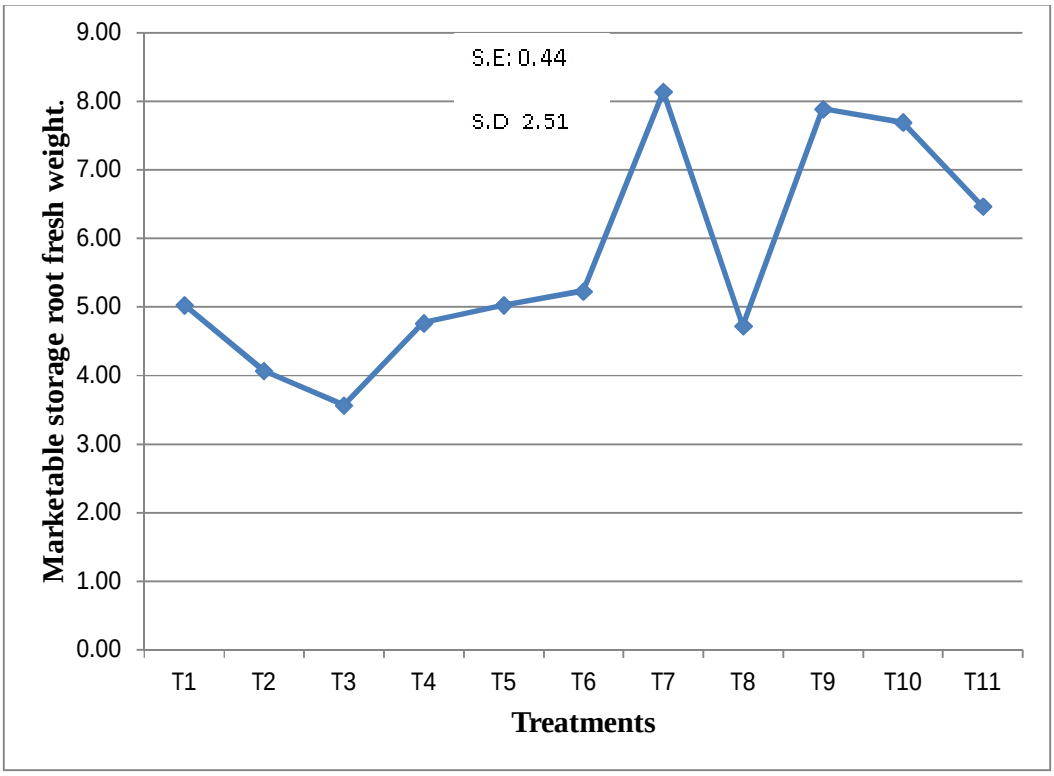


Figure (4.18). Effect of biofertilization on marketable storage roots yield fresh weight.

4.2.15 Non-marketable storage roots weight (kg)

The obtained results indicated that application of (T1) the control showed the highest yield of non-marketable yield, followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*. Meanwhile the nonmarketable yield lowest value was observed with application of (T3) *Flavobacterium* spp figure (4.19) inoculation with *Flavobacterium* showed positive effect on quality of sweetpotato storage roots yield, by reducing the non-marketable storage roots yield, but the difference is not statistically significant. In support to the current result, Muluberhan (2005) reported that non-marketable tuber yield was not significantly affected by different N rates.

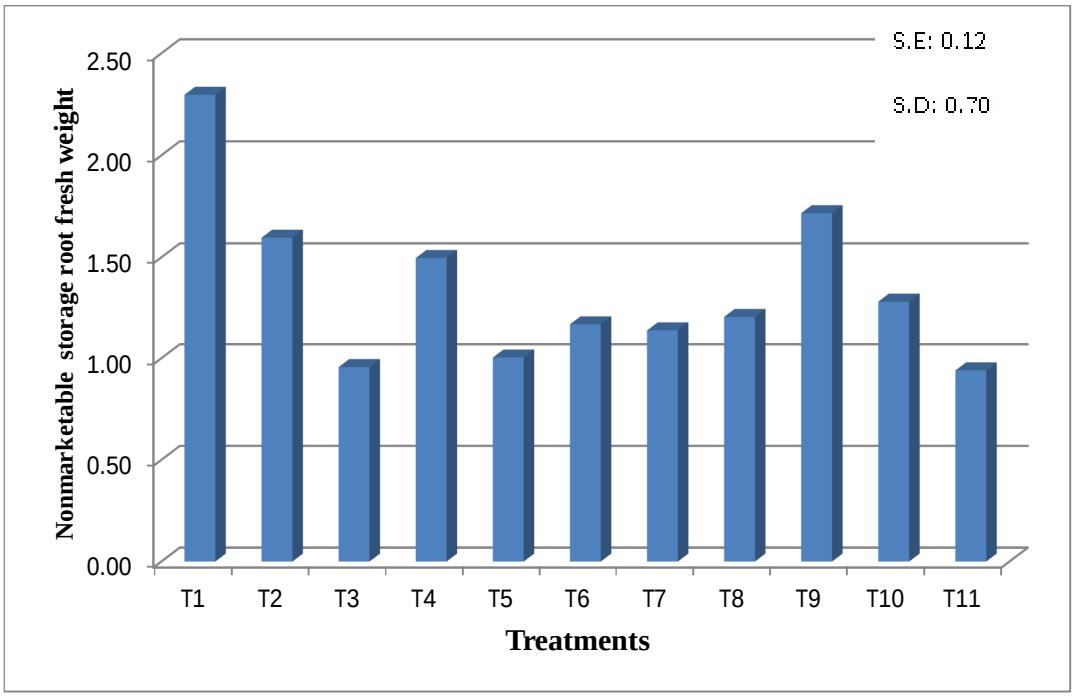


Figure (4.19). Effect of biofertilization on non marketable storage roots yield fresh weight(kg).

4.2.16 Specific gravity of sweetpotato storage roots

The obtained results showed that sweetpotato storage roots specific gravity was the highest with application of (T3) *Flavobacterium*, followed by application of (T4) 0.8t/ha of Al Khaseeb organic fertilizer, and application of (T1) the control, but statistically no significant difference. However the least value for storage roots specific gravity was obtained with application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer figure (4.20). Generally there is decrease in sweetpotato storage roots specific gravity with biofertilization, similar results were reported by (Zelalem, *et al.*, 2009) who found a decrease in specific gravity of potato in response to N application.

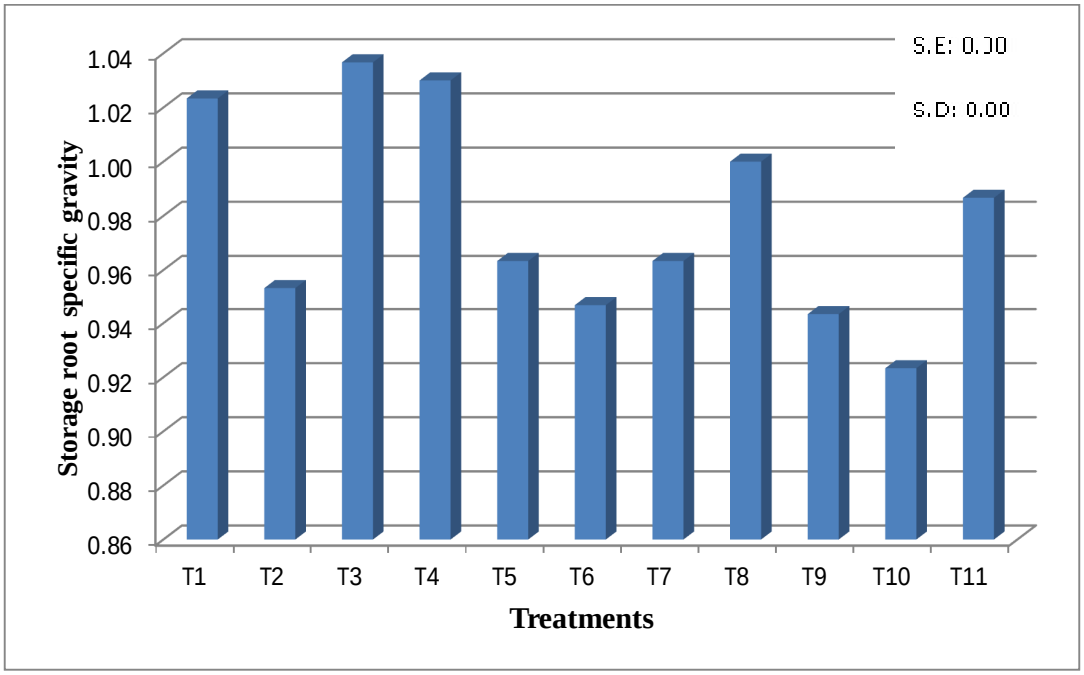


Figure (4.20). Effect of biofertilization on sweetpotato storage roots specific gravity(g/ml)

4.2.17 Sweetpotato shoots total N, Crude protein and organic carbon content (%)

The obtained results revealed that the sweetpotato shoots total nitrogen, crude protein and organic carbon contents were greatly affected by application of (T2) *Azospirillum* alone, followed by application of (T4) 0.8t/ha of Al Khaseeb organic fertilizer, and application of (T1) the control. However the lowest value was obtained with application of (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer, table (4.1), this may be due to the low organic fertilizer dose with the need of co-inoculation to improve nutrient's contents. In line with these findings Fatima, *et al.*, (2008) who reported that "Maximum value of crude protein (11.50%) was obtained by application of aqueous extract of compost at 15% + inoculation with both nitrogen-fixer strains and *B. circulans*, compared to 8.06% for controls plants This can be assigned to direct effects of bacteria on root growth, Phytohormones production, greater mineral uptake and transfer of nitrogen to the plant.

Table (4.1) Effect of biofertilization on shoots total N, crude protein and organic carbon (%):

Treatments	Total N	Crude Protein	Organic Carbon
Control.	1.28	8.00	1.67
Azospirillum brasilense	1.74	10.90	2.27
Flavobacterium spp.	1.03	6.42	1.21
0.8t/ha of Al Khaseeb organic fertilizer.	1.64	8.17	2.13
Azospirillum +0.8 t/ha of Al Khaseeb organic fertilizer.	0.28	1.77	0.37
Flavobacterium+0.8 t/ha of Al Khaseeb organic fertilizer.	0.23	1.31	0.30
Azospirillum+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer.	0.20	0.88	0.26
1.2 t/ha of Al Khaseeb organic fertilizer.	0.76	4.77	0.99
Azospirillum+1.2t/ha of Al Khaseeb organic fertilizer.	1.17	7.30	1.52
Flavobacterium+1.2 t/ha of Al Khaseeb organic fertilizer.	0.75	4.69	0.97
Azospirillum+ *Flavobacterium* +1.2 t/ha of organic fertilizer.	0.97	6.06	1.25
S.E:	**0.17**	**1.02**	**0.22**
S.D:	**0.55**	**3.23**	**0.71**

4.3 Field experiment 2 (University of Gadarif)

A. Sweetpotato pIant growth components six weeks after planting (6WAP).

4.3.1 Stem length (cm)/plant after (6 WAP)

Obtained results indicated that application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer, showed significant (P<0.05) effect on sweet-potato plants stem Length, followed by application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer and application of (T3) *Flavobacterium*. However the treatments showed very good response with the least effect on stem length for application of (T1) the non-inoculated control figure (4.21)this results indicates to the pIant growth improvement due to inoculation with PGPR similar results were obtained by Farzana, *et al*., (2007),who reported that, PGPR inoculation improved the growth parameters compared to non-inoculated Control.

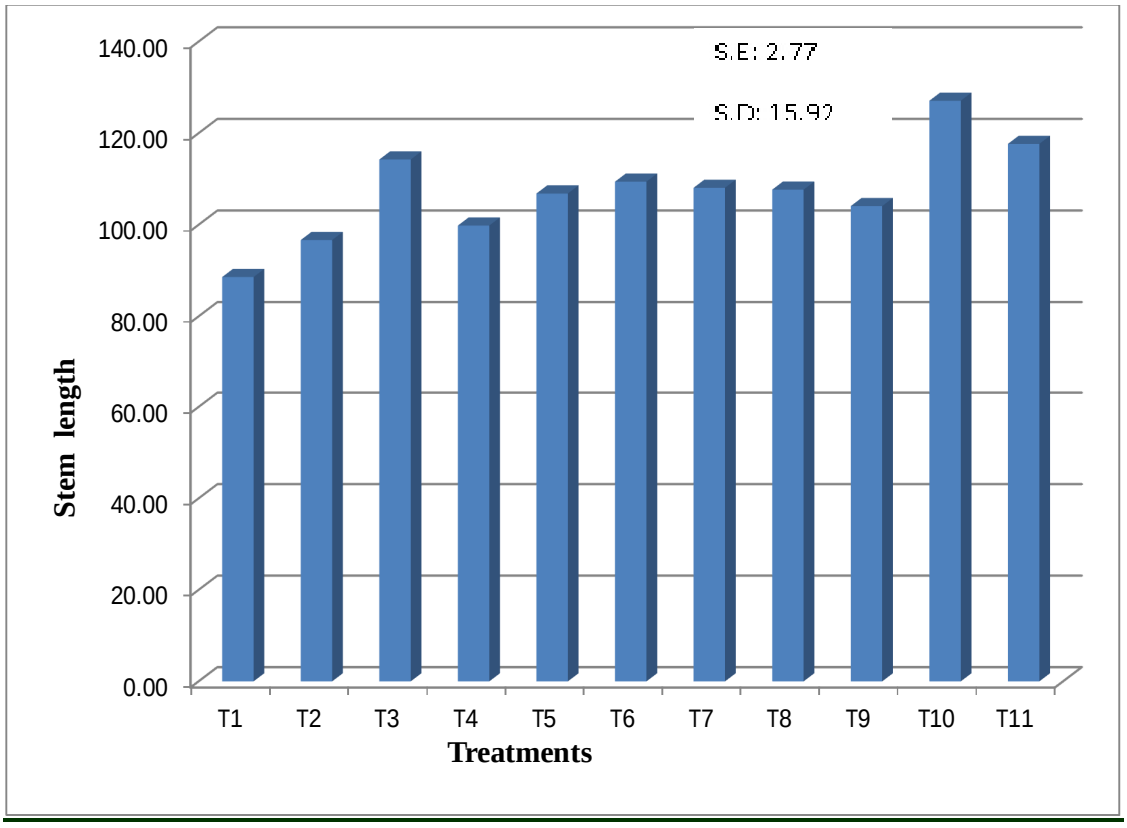
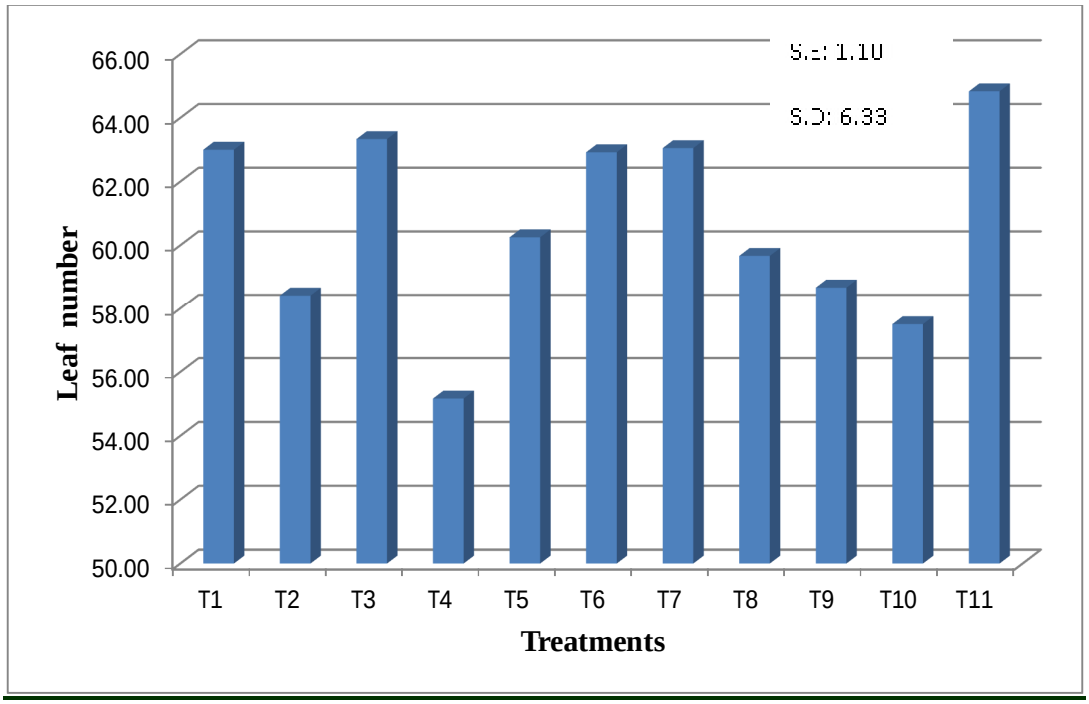


Figure (4.21). Effect of biofertilization on stem length (cm/plant) (6WAP).

4.3.2 Leaf number / plant after (6 WAP)

The obtained results revealed that application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer showed the most higher effect on sweetpotato plants leaf number six weeks after planting, but the difference was statistically non-significant ($P < 0.05$)followed by application of (T3) *Flavobacterium* spp. However application of (T7) *Azospirillum* + *Flavobacterium* + 0.8 t/ha of organic fertilizer, (T6) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer and application of (T1) the control showed almost similar effect on Leaf number meanwhile the least effect on Leaf number was observed with application of (T4)0.8t/ha of Al Khaseeb organic fertilizer alone figure (4.22) in support to this results, Zelalem *et al,*. (2009) and Mukhtar, *et al*., (2010) found non-significant increase in stem number per plant of potato in response to fertilization. This could be because this trait is much more influenced by the inherent characteristics of the crop than application of fertilizers.



Figure(4.22). Effect of biofertilization on leaf number (6WAP).

4.3.3 Branches number/ plant after (6 WAP)

Obtained results indicated that application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer showed non-significant($P<0.05$), effect on sweetpotato plants branches number at six weeks after planting, followed by application of (T6) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer. However application of (T5) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer, and application of (T4) 0.8t/ha of Al Khaseeb organic fertilizer showed similar effect on branches number. Meanwhile the least branches number was observed with the application of (T3) *Flavobacterium* spp alone figure (4.23) similarly Zelalem, *et al.* (2009) and Mukhtar, *et al.* (2010) found non-significant increase in stem number per plant of potato in response to fertilization.

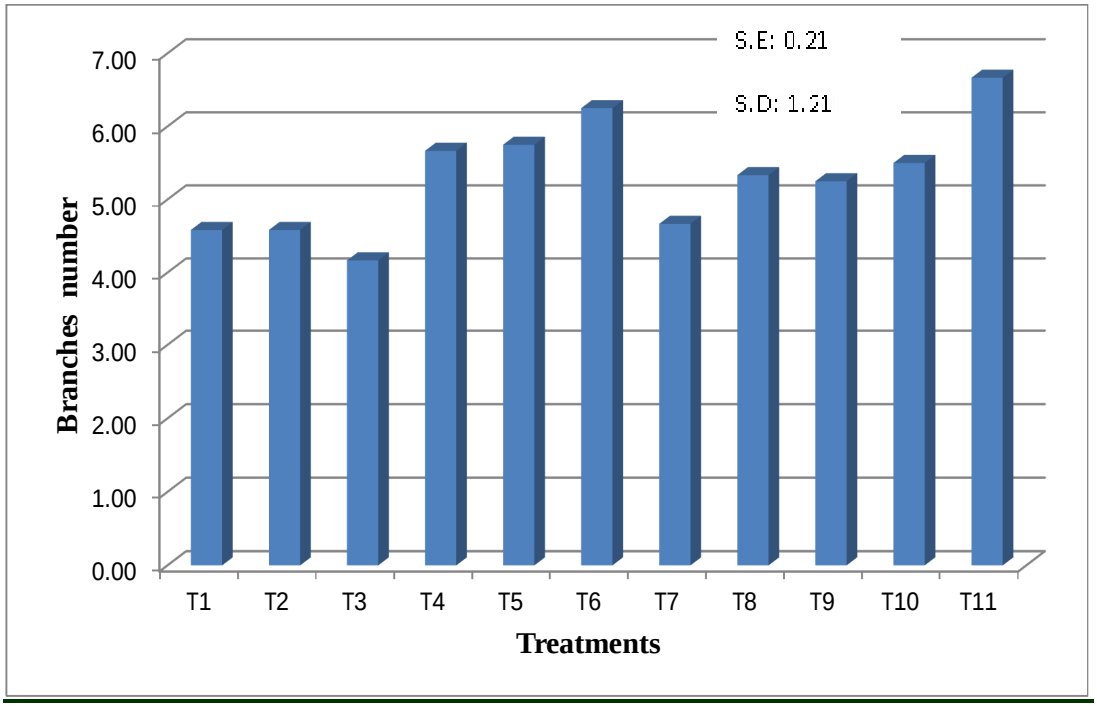


Figure (4.23). Effect of biofertilization on branches number (6WAP).

B. Sweetpotato plants growth components sixteen weeks after planting (16WAP):

4.3.4 Stem length (cm/plant) after (16 WAP)

Obtained results revealed that application of (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer, showed significant (P<0.05), effect on sweetpotato plants stem length at sixteen weeks after planting, followed by application of (T8) 1.2 t/ha of Al Khaseeb organic fertilizer. However application of (T3) *Flavobacterium* spp and application of (T11) *Azospirillum* + *Flavobacterium* +1.2 t/ha of organic fertilizer showed almost similar effect on stem length. Meanwhile the lowest stem length value was obtained with application of (T1) the control figure (4.24), similar results were obtained by Farzana, *et al*., (2007).

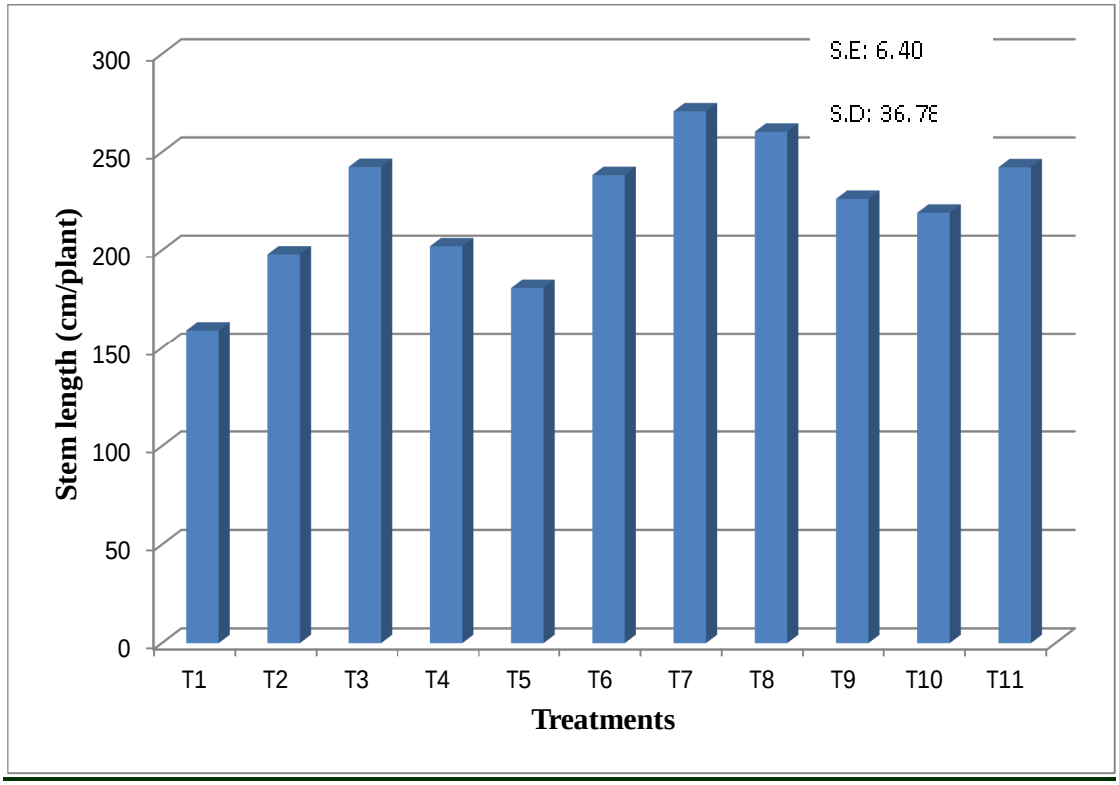


Figure (4.24). Effect of biofertilization on stem length (cm/plant)(16WAP)

4.3.5 Leaf number / plant after (16 WAP)

Obtained results indicated that application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer showed significant (P<0.05), effect on sweetpotato plants leaf number at sixteen weeks after planting, foIIowed by application of (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer and (T4) 0.8t/ha of Al Khaseeb organic fertilizer with almost similar effect on leaf number. Meanwhile the lowest leaf number value was observed with application of (T3) *Flavobacterium* spp figure (4.25) similar results were obtained by (Bashan, *et al.*, 2004).

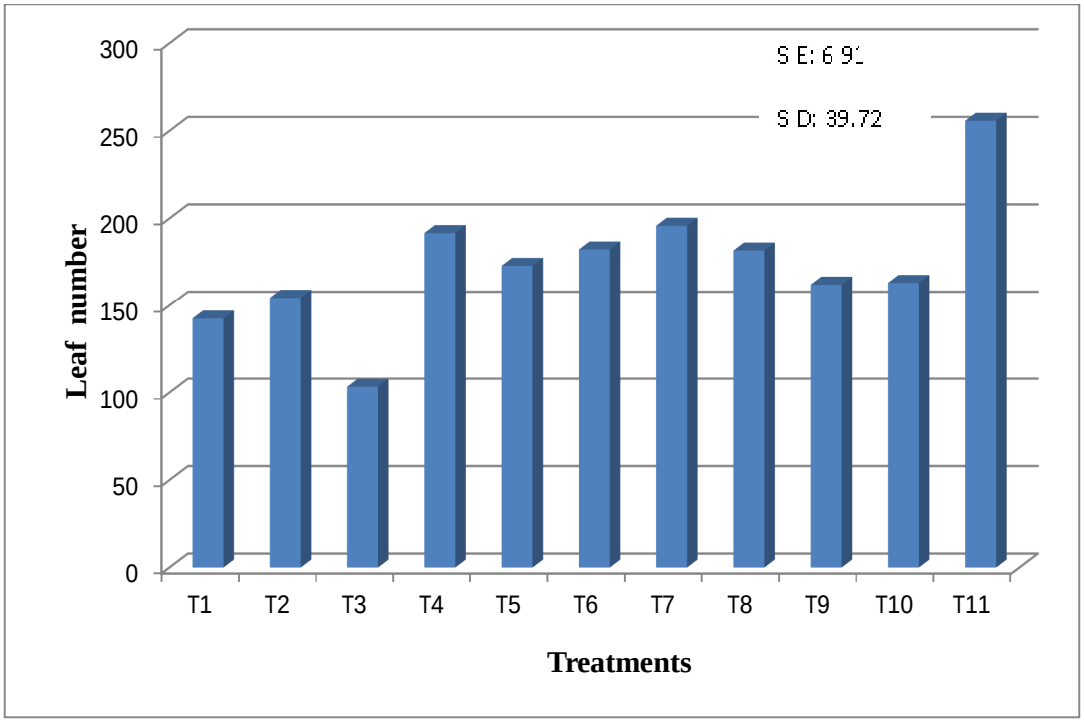


Figure (4.25). Effect of biofertilization on leaf number (16WAP).

4.3.6 Branches number / plant after (16 WAP)

The obtained results showed that application of (T5) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer, gave significant (P<0.05), effect in number of branches of sweetpotato plants at sixteen weeks after planting, followed by application of (T6) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer and application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of Al Khaseeb organic fertilizer with almost similar effect on branches number of sweetpotato plants. Meanwhile the lowest branches number value was obtained by application of (T3) *Flavobacterium spp*. figure (4.26) in contrast to this results, Zelalem, *et al*.,(2009) and Mukhtar, *et al*., (2010) found non-significant increase in stem number per plant of potato in response to fertilization.

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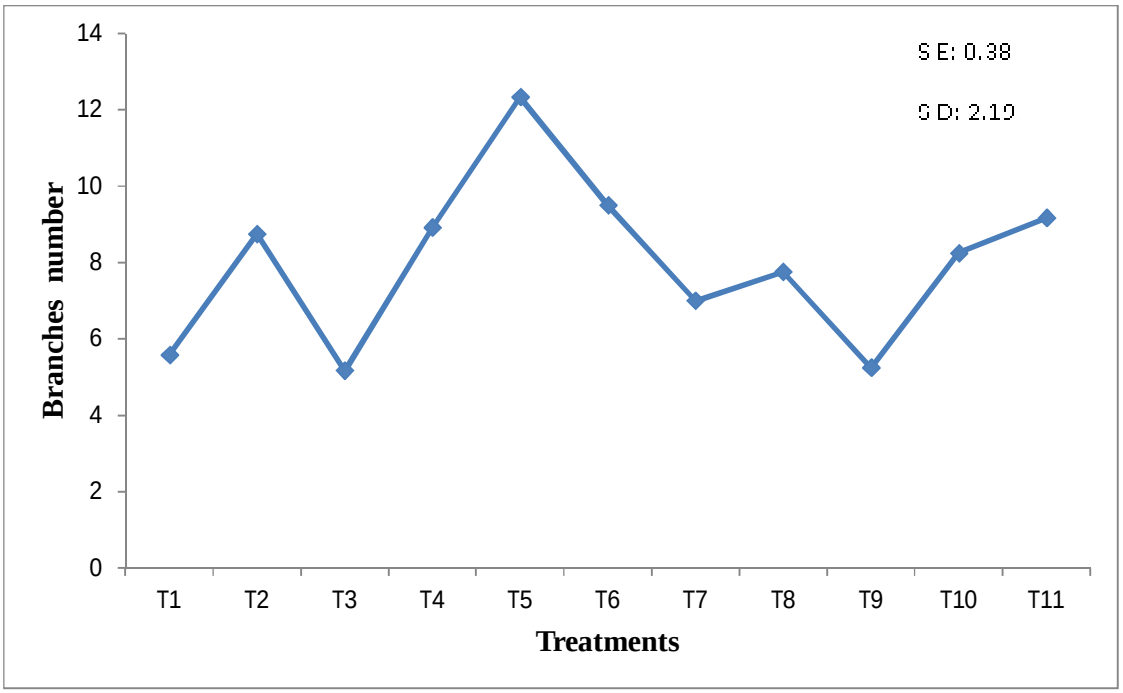

Figure (4.26). Effect of biofertilization on branches number (16WAP).

C. Sweetpotato yield components at harvesting in experiment 2 University of Gadarif “Tawawa site”:

4. 3.7 Total shoot fresh weight (kg)

The obtained results showed that the higher sweetpotato shoot fresh weight was observed with application of (T1) the control foIIowed by application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer. However application of (T7)*Azospirillum* + *Flavobacterium*+ 0.8 t/ha of organic fertilizer, application of (T6) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer and application of (T5) organic fertilizer 0.8 t/ha+ *Azospirillum* showed the almost similar effect on shoot fresh weight at site 2 experiment. Application of (T2) *AzospiriIIum* alone showed the least shoot fresh weight value figure (4.27) high soil N content highly increased he vegetative the growth and suppressed effectiveness of biofertilization, similar results were obtained by Halvin, *et al*., (2003) who reported that” increased vegetative growth through increasing cell division and elongation to be ascribed to availability of nutrients in the soil for uptake by plant roots”.

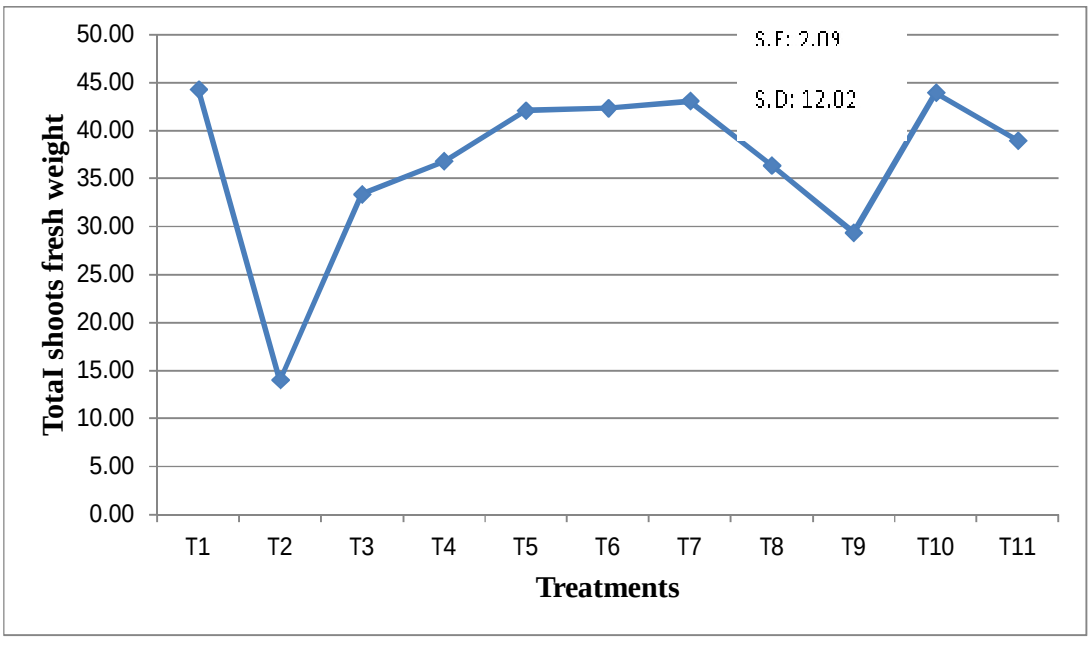

Figure **(4.27).** Effect of biofertilization on total shoots fresh weight at harvest (kg/ pIot).

4. 3.8 Shoots dry matter percentage

The obtained results showed that sweetpotato shoot dry matter content percentage was the most high with application of (T1) the control, foIIowed by (T2) *AzospiriIIum* alone, (T7) *Azospirillum*+ *Flavobacterium*+ 0.8 t/ha of organic fertilizer, and (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer. However most of the treatments showed high dry matter percentage with the lowest dry matter content with application of (T6) *Flavobacterium*+0.8 t/ha of Al Khaseeb organic fertilizer, figure (4.28) the previous fertilization history and high soil N content highly affected the effectiveness of biofertilization and this can be the most suitable interpretation for a such result.

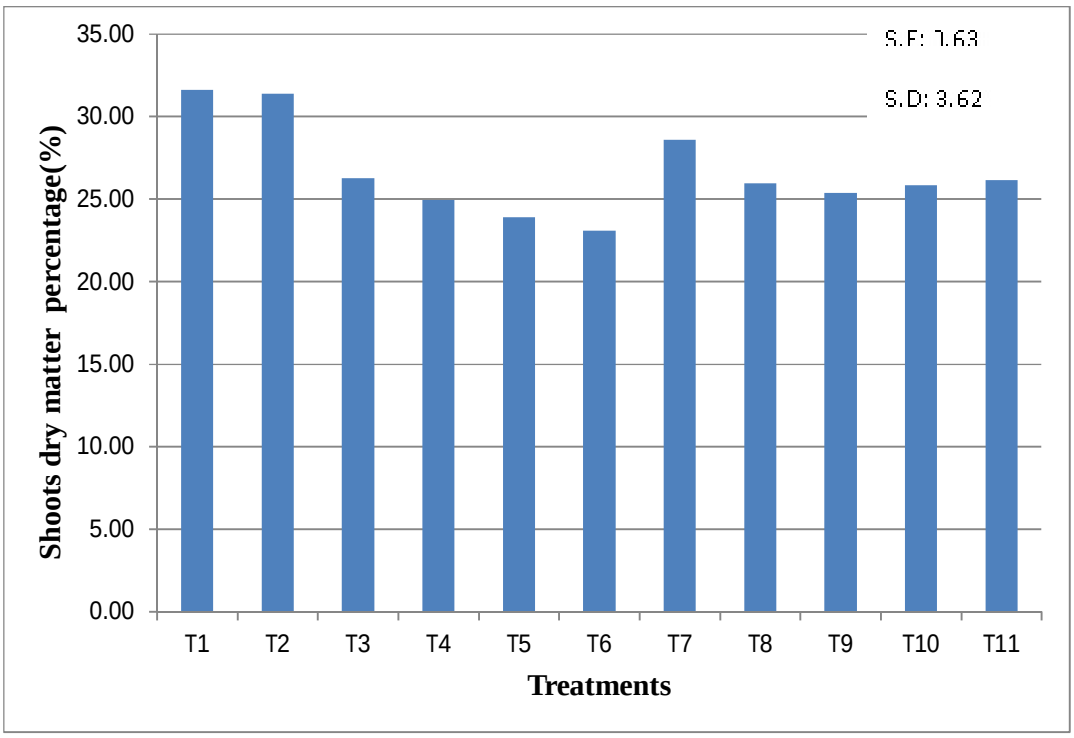

Figure (4.28). Effect of biofertilization on shoots dry matter percentage (%).

4. 3.9 Sample storage roots yield

The obtained results of sample sweetpotato storage roots fresh yield showed that application of (T10) *Flavobacterium*+1.2 t/ha of Al Khaseeb organic fertilizer, showed the highest sample yield of storage roots, followed by application of (T5) *Azospirillum* + 0.8 t/ha of Al Khaseeb organic fertilizer, and application of (T9) *Azospirillum* + 1.2 t/ha of Al Khaseeb organic fertilizer and the application of (T8) 1.2 t/ha of Al Khaseeb organic fertilizer. Meanwhile the lowest yield of storage roots was observed with application of (T2) *Azospirillum* alone, figure (4.29) this results are in line with findings of Farzana, et al.,(2007) who reported that, PGPR inoculation and Nitrogen fertilization rate significantly (P<0.05)increased storage root yield .

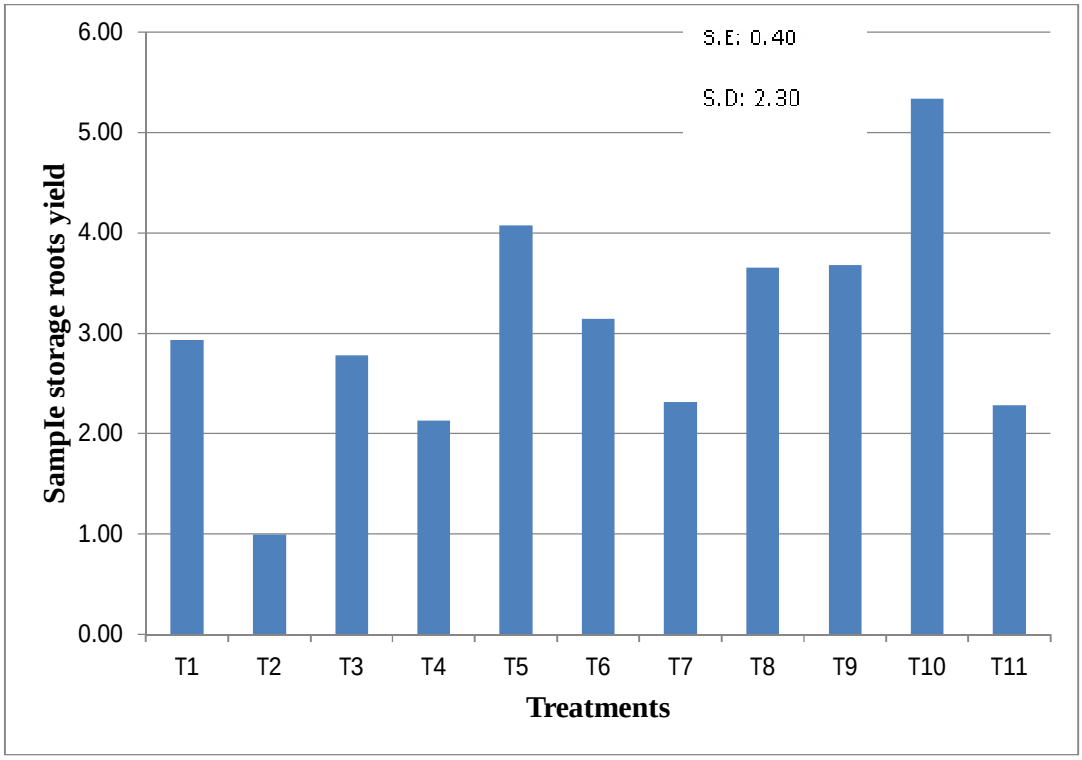

Figure (4.29). Effect of biofertilization on sample storage roots yield (kg).

4. 3.10 Total storage roots yield (kg/ plot)

Results obtained showed that total sweetpotato storage roots yield was highly affected by application of (T10) organic fertilizer 1.2 t/ha+ *Flavobacterium*, followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, and application of (T5) organic fertilizer 0.8 t/ha+ *Azospirillum*. The yield of control was also high and this may be due to the history of pervious fertilization in site 2. Optimum storage roots yield was obtained with combined application of (T10) organic fertilizer 1.2 t/ha+ *Flavobacterium*, followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, figure (4.30),this results are in line with findings of Getu (1998), Girma (2001) and Yibekal (1998) who reported that N levels significantly increased marketable tuber yields of potato.

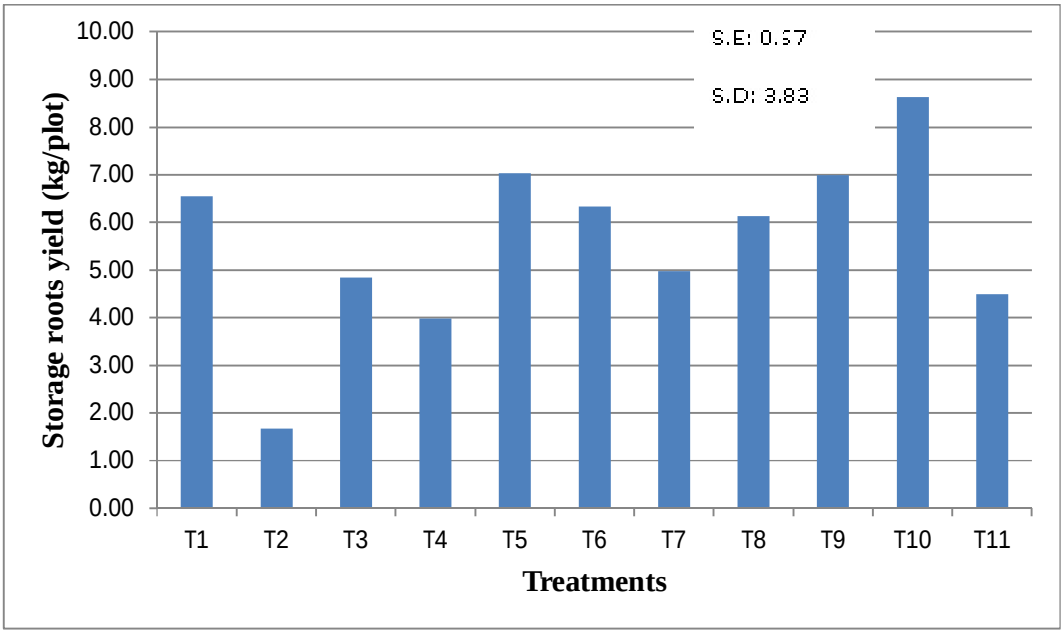

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Figure (4.30). Effect of biofertilization on total storage roots yield (kg/ plot).

4. 3.11 Storage roots dry matter percentage

The obtained results showed that sweetpotato storage roots dry matter percentage was the most higher by application of (T10) organic fertilizer 1.2 t/ha+ *Flavobacterium*, foIIowed by application of (T11) *Azospirillum*+ *Flavobacterium* +1.2 t/ha of organic fertilizer and (T4) organic fertilizer 0.8 t/ha alone. However most of the treatments showed high dry matter percentage, but statistically no significant difference among treatments figure (4.31) similar results were obtained by Sparrow, *et al.*, (1992) who observed non-significant effect on percent dry matter of tubers due to increased P application.

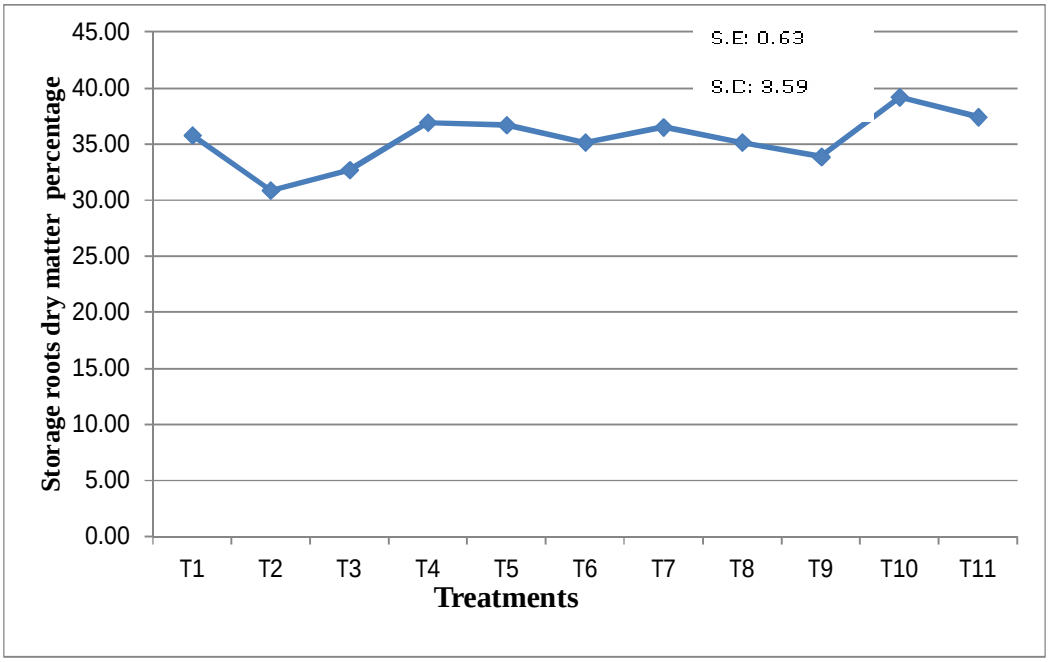

Figure (4.31). Effect of biofertilization on storage roots dry matter percentage

4. 3. 12 Total storage roots yield (t/ha)

Obtained results showed that sweetpotato storage roots yield t/ha was high with application of (T10) organic fertilizer 1.2 t/ha+ *Flavobacterium,* followed by application of (T9) organic fertilizer 1.2 t/ha+ *Azospirillum*, and application of (T5) organic fertilizer 0.8 t/ha+ *Azospirillum*. The yield of control was also high and this may be due to the history of pervious fertilization in site 2 "Tawawa". However the least value for total storage roots yield was obtained by application of (T2*) Azospirillum brasilense* inoculation alone indicating to the importance of organic fertilizer and co inoculation figure (4.32) similar results were reported by Gravel, *et al.*,(2007), Kozdroja, *et al.*,(2004) and Shaharoona, *et al.*, (2006),when they reported " similar promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR.

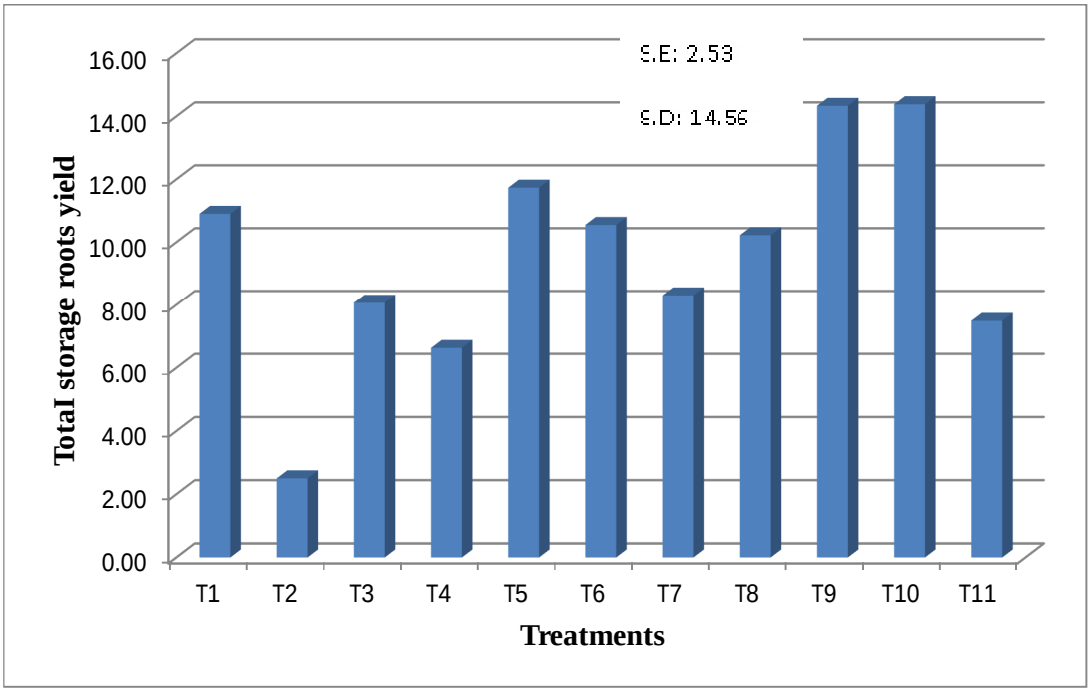

Figure (4.32). Effect of biofertilization on total storage roots yield (t/ha).

4. 3. 13 Marketable storage roots yield (kg/ plot)

Obtained results showed that the quality of storage roots yield and marketable storage roots yield, was significantly affected by application of (T10) *Flavobacterium* +1.2 t/ha of organic fertilizer in site2, followed by application of (T5) organic fertilizer 0.8 t/ha+ *Azospirillum* and application of (T9) *Azospirillum* +1.2 t/ha of Al Khaseeb organic fertilizer. However the adverse effect on marketable storage roots yield was with application of (T2) *Azospirillum* alone showing the lowest value, and co-inoculation with reasonable rate of organic fertilizer is the best figure (4.33), similarly Hameeda, *et al*. (2007) found that the application of microbial inoculants along with higher concentrations of composts may not be synergistic for sorghum plant growth.

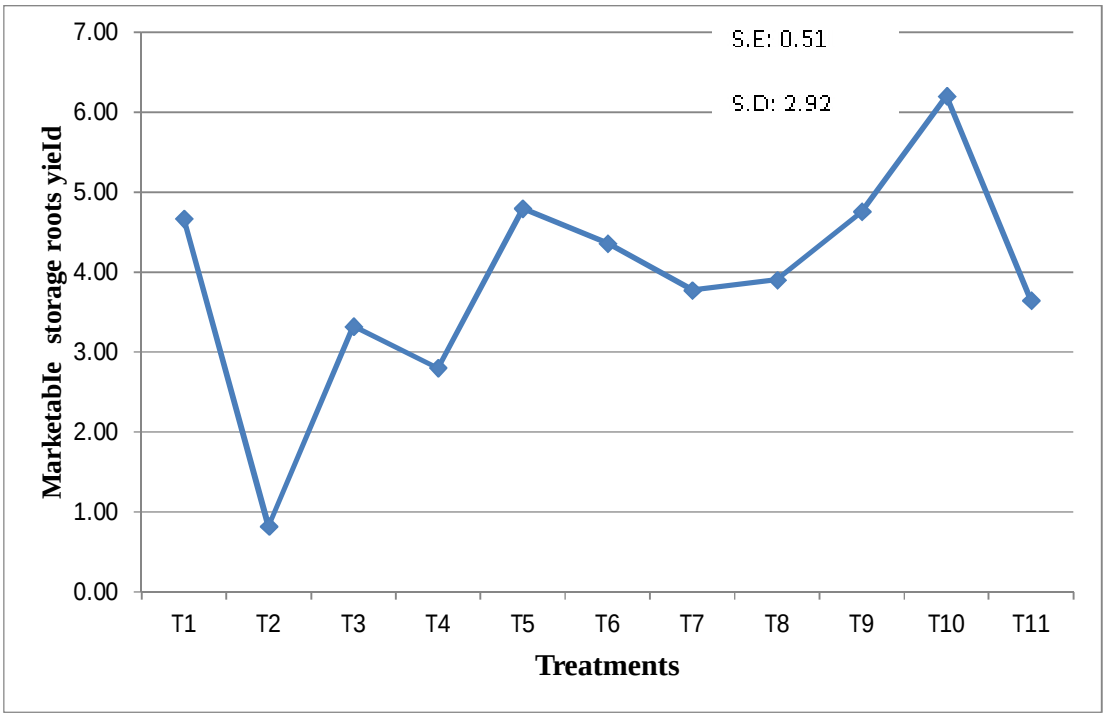

Figure (4.33). Effect of biofertilization on marketable storage roots yield (kg/plot).

4. 3. 14 Non-marketable storage roots yield (kg/ plot)

The obtained results indicated that application of (T10) *Flavobacterium* +1.2 t/ha of organic fertilizer, showed the highest yield of nonmarketable storage roots yield, followed by application of (T5) *Azospirillum*+ organic fertilizer 0.8 t/ha. Meanwhile the nonmarketable yield lowest value was obtained with application of (T2) *Azospirillum* alone; figure (4.34) similar results were obtained by Teshome,(2012) who reported that ” the interaction effect of farmyard manure and phosphorus did not influence both total and unmarketable tuberous root yield.

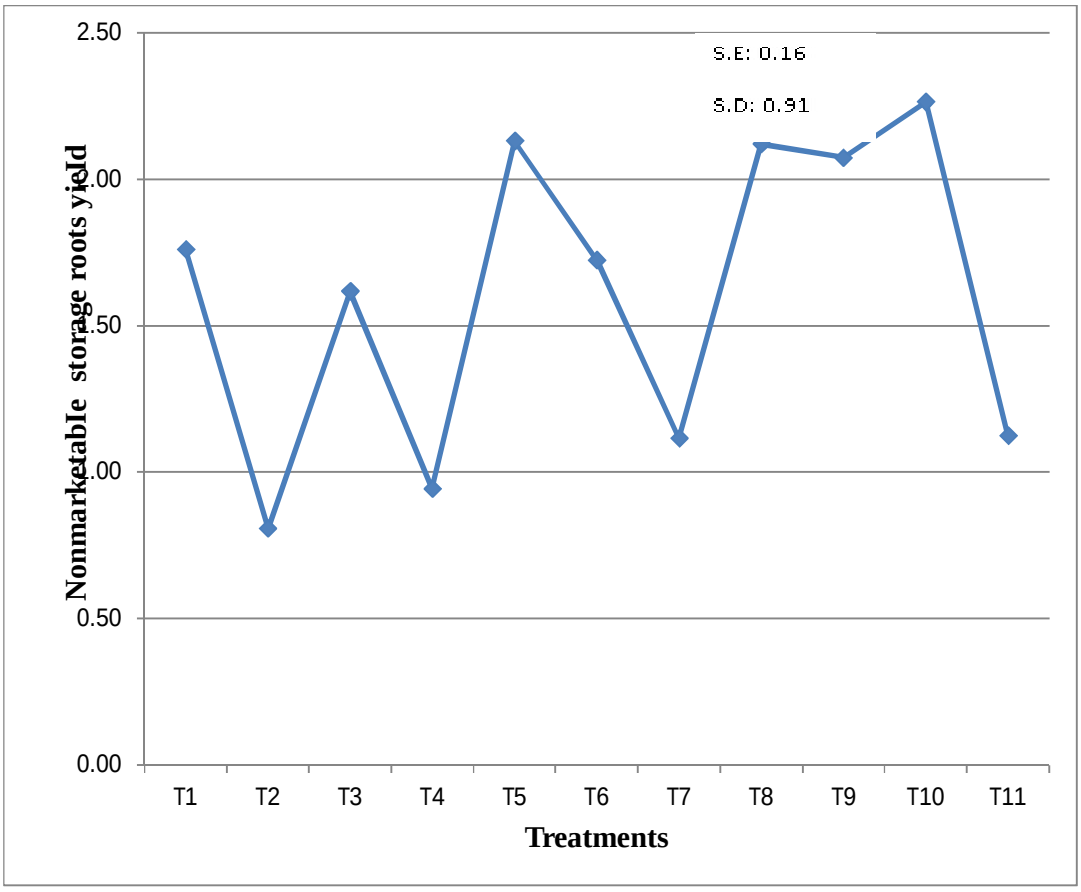

Figure (4.34). Effect of biofertilization on non marketable storage roots yield.

4. 3. 15 Storage roots specific gravity

The obtained results showed that storage roots specific gravity as indicator for the sweetpotato storage roots quality was the highest with application of (T11) *Flavobacterium* + *Azospirillum*+1.2 t/ha of Al Khaseeb organic fertilizer, followed by application of (T3) *Flavobacterium* alone. However the least value for storage specific gravity was obtained by application of (T2) *Azospirillum* alone, this can indicate to the importance of co-inoculation and the application of organic fertilizer for effective biofertilization figure (4.35) in line with this results (Zelalem, *et al*., 2009) found a decrease in specific gravity of potato in response to N application from 0 to 207 kg ha-1 at vertisol of Debre Berhan area.

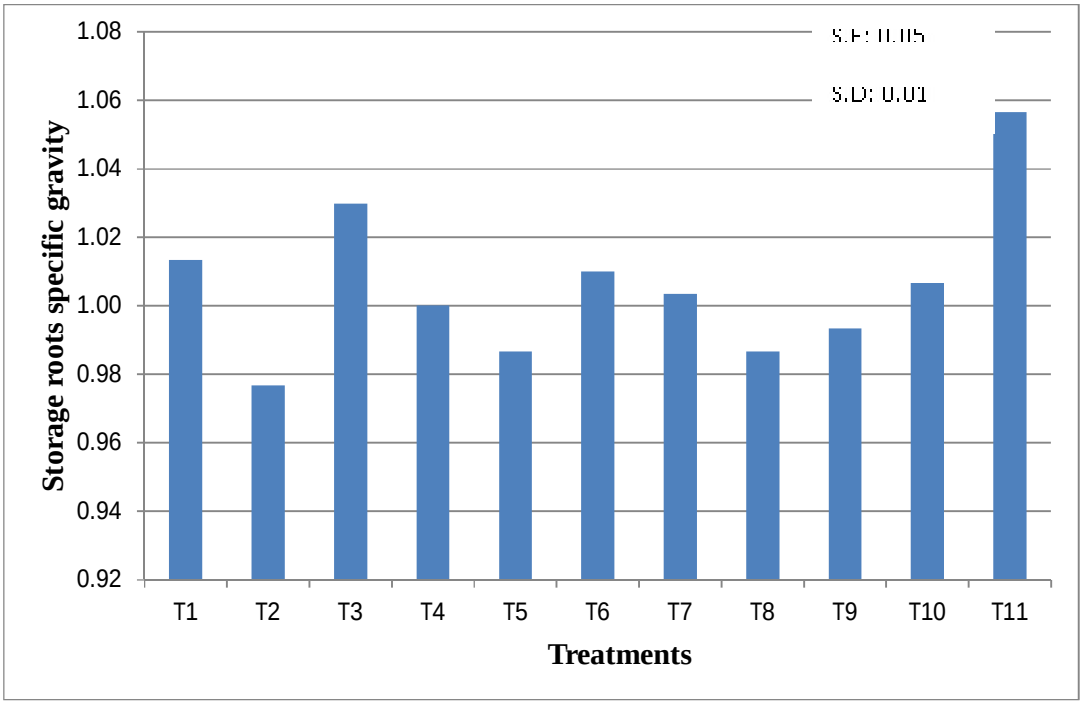

Figure (4.35). Effect of biofertilization on sweetpotato storage roots specific gravity.

CHAPTER FIVE

CONCLUSIONS and RECOMMENDATIONS

Generally the crop showed very good performance with the biofertilization, and there is a great potential of growing 100% organic sweetpotato under field conditions in Sudan. Some plants storage roots formations grew up to 2.2 kg/plant and this can be ideal in such soil conditions. By more improving soil compaction, plots size and the plot height, the yield could be greatly improved. The sweetpotato cultivar showed considerable resistance to sweetpotato weevil and other pests; with high yield and good storage roots color and shape. Shoots vegetative growth could be an indicator for a high yield of storage roots referring to the important role of a good crop vegetative establishment in the crop yield. However in some cases the high vegetative growth can be indicator of excess nitrogen in the soil or over dose fertilization and this can suppress the effectiveness of the biological nitrogen fixation process and effectiveness of microbial inoculants such as *AzospiriIIum* and *FIavobacterium*, it's believed that the microbial Inoculants have many mechanisms t o improve plants growth such as plant growth hormone production, this findings are in line with findings of Desmond and Walter (1990)when they" suggest that the inoculation with *Azospirillum* contributes to sweetpotato root growth by mechanisms other than supplying N; stimulation of growth by growth hormones, as noted earlier, may be one of such mechanisms". Also the extra vegetative growth can be at the expense of the storage roots formation.

Through the biofertilization more cheap, healthy and safe sweetpotato and other crops could be produced with conservation of the environment and natural resources. As general the crop is sensitive to water logging and more

care is needed in soil leveling during soil preparation stage. The study showed that in the area with pesticide history no storage roots infestations were observed. By exploiting the orange fleshed sweetpotato we can improve human nutrition, combat hidden hunger (nutrients imbalances in food) and reduce or eliminate the Vitamin A deficiency (VAD), beside saving millions of dollars for the national economy.

The crop vegetative growth parameters showed increase and the plants were creeping and the shoots growth was reasonable with alot of flowering plants during the experiment period in experiment 1 in Shambat site. However the crop vegetative growth parameters showed great increase and the plants were almost semi Erect and the shoots growth was very high with few Flowering plants in experiment 2 in Tawawa site, but the storage roots yield was on reverse; as it showed good sweetpotato storage roots yield with reasonable vegetative growth in site 1 experiment. Mean while the sweetpotato storage roots yield decreased when the vegetative growth was very high indicating to excess soil nitrogen and less effectiveness for the inoculation with the biofertilizers, from these results we can conclude that the same variety can behave differently in different environmental conditions or different locations.

The yield of sweetpotato storage roots in experiment 2 University of Gadarif site was quite low compared to experiment 1College of Agricultural studies (CAS), Sudan University of Science and Technology, Shambat site. aIso high vegetative growth can be encouraged by cold weather conditions, and prolonged vegetative growth period can be on the expense of storage root formation and the total yield. Many factors can be among the reasons for such differences between the two sites, including Soil type and Texture and irrigation water and it's availability , but the crop performance was as general was very good. However there was full absence for storage roots

infestation in experiment 2University of Gadarif site; this may be due to soil chemical and physical properties.

Most of the people in Sudan lacks awareness about the benefits of orange fleshed sweetpotato and it's nutrients contents, and therefore they prefer Irish Potato on white fleshed sweetpotato. Great efforts are needed to convince the people to substitute the white fleshed sweetpotato and potato with the orange fleshed sweetpotato for it's benefits and nutrients contents; and this role should be played by the extensional services.

Orange fleshed sweetpotato is very nutritive and healthy food source and Initiation of National Research Program on Orange fleshed sweetpotato to improve human nutrition and combat vitamin A deficiency (VAD), in infants and Lactating women specially and all the citizens as general; is highly recommended and urgently needed.

Conduction of further research with improvement of the soil and water conditions is of great importance and highly recommended.