

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies



**Effect of Rhizobium, Mycohriza and Diammonium
Phosphate fertilizer on the Nodulation, Growth and Yield of
Chickpea (*Cicerarietinum L.*).**

تأثير البكتريا العقدية والمايكورايزا و الفوسفات ثنائي الامونيوم في العقد
الجزرية ومعدل النمو و الانتاجية لمحصول الحمص.

Athesis Submitted in Partial Fulfillment for the Requirements
for the Degree of M.Sc. (Agronomy).

BY:

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Supervisor:

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September 2015

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الآية

قال تعالى:

الدُّنْيَا وَآكَافِهَا (بِأَيِّ آيَةٍ أَنْزَلْنَا لَهُ مِنْ السَّمَاءِ مَاءً فَآخَذَتْهَلَطَ بِهِ نَبَاتٌ
صَبِيحًا هَشِيمًا تَذُكَّرُ بِهِ أُولُوا الْأَلْبَانِ يُعَالَجُ كُلُّ شَيْءٍ مِمَّا تَدْرَأُ)

صدق الله العظيم

سورة الكهف الآية (45)

DEDICATION

Thesis study dedicated to my great father, my lovely mother, my brothers & sisters, my friends & colleagues.

To all who have been helpful, tolerant and patients and offered me unconditional assistance in performing this task and whom without their great assistance I could have not to accomplish this achievement.

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ABSTRACT

Single season field experiment was carried out in the Food Security Farm River Nile State at Atbara in the winter season (2014). The experiment was aimed to study the effect of rhizobium, mycorrhiza and diammonium phosphate fertilizer on the nodulation, growth and yield of Chickpea. The experiment a randomized complete block design was used with four replicates for this purpose. The treatments were: control, inoculum, inoculum + mycorrhiza, inoculum + mycorrhiza + DAP 120 kg /ha and inoculum + mycorrhiza + DAP 140 kg /ha.

The treatment rhizobium + mycorrhiza resulted in the highest relative growth rates, number of nodules, and grains yield t/ha with statistically significant differences over the other treatments. But the treatment rhizobium gave significant difference in 100 seeds weight (gm), however the treatment control and rhizobium + mycorrhiza + DAP 140 kg /ha gave the highest number of pods/plant significantly differences over the other treatments. But the treatment rhizobium + mycorrhiza + DAP 120 kg /ha gave the highest number of seeds/pod.

الخلاصة

أجريت تجربة حقلية لموسم واحد بمشروع الامن الغذائي بولاية نهر النيل بمدينة عطبرة في شتاء 2014 لدراسة تاثير البكتريا العقدية ، المايكورايزا و سماد فوسفات ثنائي الامونيوم على تكوين العقد البكتيرية ، معدل النمو و انتاجية الحمص.تم استخدام تصميم القطاعات الكاملة العشوائية بأربع مكررات و كانت المعاللات كالاتي: الشاهد، لقاح عقدين،لقاح عقدين + مايكورايزا، لقاح عقدين + مايكورايزا + سماد فوسفات ثنائي الامونيوم120 كجم للهكتارولقاح عقدين + مايكورايزا + سماد فوسفات ثنائي الامونيوم140 كجم للهكتار.

أظهرت نتائج تفوق المعاملة لقاح عقدين + مايكورايزاعلى باقي المعاللات بفروقات معنوية في معدل النمو، عدد العقد الجزرية بالنبات و الانتاجية على باقي المعاللات. كذلك معاملة لقاح عقدين أعطت فروقات معنوية في وزن المائة بذرة (جم) على باقي المعاللات. بينما معاملة الشاهد و لقاح عقدين + مايكورايزا + سماد فوسفات ثنائي الامونيوم140 كجم للهكتار تفوقا في عدد القرون في النبات الواحد، أما لقاح عقدين + مايكورايزا + سماد فوسفات ثنائي الامونيوم120 كجم للهكتار أظهرت اعلى النتائج في عدد البذور في القرن.

CHAPTER ONE

INTRODUCTION

Chickpea (*Cicer arietinum L.*) belong to genus cicer, family Fabaceae. Is an according to (Ducke, 1981), chickpeas were first domesticated in the Middle East and was widely cultivated in India, Mediterranean area , the Middle East, and Ethiopia spread to the new world, it is now important in Mexico, Chile, Peru and the U.S. and important in Australia. Wide spices are also most abundant in Turkey, Iran, Afghanistan, and center Asia. Chickpea also known as Bengal gram (or simply gram) and garbanzo beans and the second most important food legume crops in the world sown in 11 million ha worldwide with a total production of 9 million tons in 2006-08. South Asia is by far the largest producer chickpea (76%) in the world with a share of more than 80% of area harvested. Like dry beans, the developing world's share in total area and production of chickpea is 95% and 93% respectively. The region of Middle East and North Africa (MENA) is the second most important region of chickpea area production following by SSA. The SE Asia and LAC as a region have more than 100 thousand ha of chickpea, but the relatively insignificant players from the global perspective (Sitou, 2011).

The main producing area in the Sudan north of Khartoum specially River Nile State, Northern State and Gabal Marrah and also grown in limited area of White Nile State (Al-khidir, 2007).

In the Sudan the average harvested area 2006-08 about 0.01 million ha, percentage share in area harvested 0.07, the average production 0.01 million tons and the average yield 1.92 tons/ha (Sitou, 2011).

The mature seeds are eaten whole, or they are ground into flour, or split to make dhal and the immature pods and young leaves are used as vegetables (Al-khidir, 2007)

It is known to be salinity and drought tolerant but can grow in a rain-fed cool-weather crop or as a dry climate crop in semi-arid regions. Optimum condition

include 18-26⁰C day and 21-26⁰C night temperature and annual rainfall of 600-1000mm. Frost hailstones, and excessive rain damage the crop. Though sensitive to cold, some cultivars tolerate temperature as low as -9.5⁰C in early stage or under snow cover (Duke, 1981).

The significance of phosphorous availability for nodulation reported by El-Siekh (1993) as well as increased availability of iron and N₂-fixation. The requirements of host plants for optimal growth and symbiotic N₂-fixation processes for phosphorous have been assessed by determination of nodule development and functioning (Brundrett, 2009). In the last decades, and due to the increasing demand for legume products as well as chickpea, the production was extended towards the alkaline clay soils mainly in the Central Clay Plain of the Sudan where the land is not a limiting factor. In such soils, the degree of nodulation was monitored to be very poor (Hamid, 2005 and Gumaa, 1999). They attributed this phenomenon to the impact of the heavy texture of the clay soils mainly to the poor aeration. The poor availability of both phosphorous and iron, under the conditions of alkaline clay soils was reported hinder nodulation and fixation of Guar (Hamid, 2005). Vesicular-arbuscular mycorrhiza (VAM) was found to improve the availability of phosphorous and other immobile elements like zinc and iron (Baylis, 1959).

The objective of this study is to evaluate the effect of Rhizobium inoculums, Mycorrhiza, and Diammonium phosphate (DAP fertilizer) on: Nodulation, Growth, Yield and Primary yield components of chickpea.

CHAPTER TWO

LITERATURE REVIEW

2.1. Botanical Description:

Chickpea is an erect herbaceous annual, rarely taller than 60 cm. the whole plant covered with glandular hairs. It has strong tap root and a mass of lateral roots in the upper layer of the soil. The plant is well branched with pinnate leaves about 5cm long, with 10-20 leaflets. The flowers can be of many colors and borne singly on long maxillary peduncles. The pod is 2cm long and 1cm broad and contains 1-3 spherical. Wrinkled seeds with an oblique, pointed peak. The seeds are commonly brown, but sometimes white, red, or black. Brown or black-seeded types are more tolerant to adverse soil and climate conditions, whereas white-seeded ones although high yield, do well only under more favorable conditions. It is self pollinated crop though occasionally cross-pollination occurs. On cloudy and wet days little pollination takes place and empty pods result (Onwueme, 1991).

2.2. Utilization:

Like other legumes, Chickpea is somewhat deficient in the amino acids, methionine and cystine, but rich in lysine and tryptophan. It is useful supplement to cereal and other starchy foods. It is rated as being highly digestible, particularly the white and cream colored-seeds types. The dried Chickpea is used in many ways. The whole dried seeds are eaten boiled. Dahl is made by splitting the seed removing the seed coat. Gram flour is made by grinding the seed after the coat has been removed and is used to make many sweet and savoury dishes. The tender shoots and green pods are used as vegetables. The fresh and green grains are eaten raw and cooked as vegetables. Green Chickpea seeds are also canned. The green plants are used as forage. After harvesting the threshing, the chaff is fed to livestock. The grain is also fed to animal, especially horses and bullocks. An acrid liquid from glandular hairs of the plant is collected by spreading a cloth over the crop at the night. The cloth absorbs the exudation with the dew. The exudates contain about 94% malic acid and 6% oxalic acid and is used medicinally (Onwueme, 1991).

2.3. Adaptation:

Chickpea is adapted to cool and moderate temperature during growing period, but tolerate considerable heat during fruiting and repining period. Early summer heat shortens the growing period, hastens maturity and reduce yield. Chickpea tolerate to drought but does not grow well in warm, humid climates. In Asia countries it is sown in winter (October-November) and grown in the moisture conservation in the soil. In Asia and Mediterranean countries where chickpea is grown, where a little rainfall during the growing season. Light to moderate rainfall is good for the crop, but not heavy rainfall. Rainfall during the growth period is more beneficial than the flowering period. Frost during flowering and fruiting is very detrimental to the crop. It is grown in wide range of soils. It is not tolerate to wet soils. The most suitable soils are moderate to heavy, well determined soils, i.e. clay loams and loams. High fertility in the soil stimulates excessive vegetative growth at the expense of seeds production. Chickpea is notably tolerated to soil salinity (Al-khidir, 2007).

2.4. Fertilization:

Chickpea does not require nitrogen when natural or artificial inoculated with root nodule bacteria. Inoculating seeds with specific strain of rhizobium bacteria that lead to appear increasing in yield, especially when the growing crop at the first time in the area or growing in an area that has been stopped growing for many seasons (fallow). The best fertilizers which added to chickpea and gave the high response are Phosphorous fertilizers. In dry season or in low rain winter season areas the fertilization is not commercially. Nitrogen fertilizers added by relatively low rate about 30-40kg/ha, phosphorous 50-80kg/ha, and potassium 40-50kg/ha. In Sudan at Alrubatab areas recommended to add 18kg N/ha but at Wed Hamid area recommended that no nitrogen fertilizer to add. In the sand soil fine texture the crop response to organic fertilization about 20-40tons/ha and added before deep ploughing. Organic fertilizers are increase the yield and protein percentage in the seeds and clearly increase the ability plant disease resistance (Al-khidir, 2007).

2.5. Presence of indigenous rhizobium:

Rhizobium strains that infect chickpea are specific and rarely exist especially in the soil which chickpea was currently grown. All uninoculated varieties showed no

nodules in their roots. This may indicate the absence of infective *Rhizobium* strains of chickpea inoculation. This could be support by the fact that, the studies area Atbara has not been cultivated with chickpea. These findings are in agreement with the result of Karrar, (1984) who reported that chickpea was never found to nodulate naturally in Khartoum and Tokar Delta. Also Mukhtar and Abu Naib, (1988) reported that the area in which chickpea is not a traditional crop nodulation was invoke only after nodulation with component *Rhizobium* strain. However, even in areas which chickpea may be grown such as Hudaiba and natural nodulation of the crop was very poor (Ibrahim, 1980).

2.6. Effect of some environmental factors on chickpea nodulation:

The-legume *Rhizobium* symbiosis is a highly integrated and self-regulated process, it is particularly sensitive to drought. Under drying conditions the viability of rhizobia show rapid declining moreover nodulation does not occur when the soil is dry (Al Sheikh, 1993). Soil moisture deficit is always associated with high soil temperature. Under tropical conditions, high temperature may adversely affect the survival of rhizobia on the nodulated seed in the field. High temperatures also adversely affect nodulation and nitrogen fixation. Soil pH is consider to be the most important in the determining the existence of free rhizobia. Soil pH influences N₂-fixation by direct and indirect effect on the rhizobia and the host. The effect of soil pH on nodulation and N₂-fixation can be a consequence of direct inhibition of *Rhizobium* survival, colonization and competitiveness for nodulation site. Generally pH range from 6.5 to 7 is considered optimal for growth of most *Rhizobium* strains. Saline condition may limit legume *Rhizobium* symbiosis by affecting survival and production of *Rhizobium* spp. In the soil and rhizosphere, inhibition of the infection process, directly affecting nodule function and reducing plant growth photosynthesis and demand of nitrogen (Rupela and Rao, 1987).

Observed significant decrease in nodules number per plant and total plant weight of chickpeas salinity was increased, moreover, nodulation was in hibited at 7 ds/m(El-Sheikh, 1992).

2.7. Rhizobium Inoculum:

Chickpea is generally is inoculated with *Brady rhizobium* sp cicer. The effect of *Rhizobium* inoculum on chickpea yield depends on the native rhizobal status.

Fields in which well nodulated chickpea was grown previously do not require Rhizobium inoculums. Where the chickpea is being grown after padding or inoculated for the first time, Rhizobium inoculums is necessary. A yield increase of 12% was recorded with Rhizobium inoculums alone. Rhizobium inoculums along with the recommended fertilizer application increase yield by 40% in Sabour and Bhagalpur (Atabani, 1988).

2.8. Response of chickpea to nitrogen fertilization:

Nitrogen is one of the most essential elements needed for the plant growth. It is found in many forms which urea is the second most popular carrier. When nitrogen status was low, 90% increased in seed yield of chickpea was obtained 43 kg N/ ha, while 170% increased obtained with 84 kg N/ ha (Nourai, 1987).

2.9. Phosphorus –significance to nodulation and N₂–fixation:

The improvement of phosphorous is very important in the rhizobium attachment to the root hairs and the synthesis activity of nitrogenase enzyme which need energy as ATP. The significance of Phosphorus for nodulation was also reported by Al-Shiekh (1993) as well as increased availability of iron and N₂–fixation. The nitrogenase enzyme is composed of Fe-protein and Fe-Mo-protein (Silver and Hardy, 1978). The application of 50kg P₂O₅/ha improve the nodulation process (Carroll, 2001).

2.10. The poor availability of phosphorous in alkaline clay soils:

In alkaline clay soils, the degree of nodulation was monitored to be very poor. They attributed this phenomenon to the impact of the heavy texture of the clay soils mainly to the poor aeration. The alkaline pH interferes with the availability of same essential nutrient elements e.g. P and Fe to the symbiotic N₂-fixation (Hamid, 2005 and Gumaa, 1999).

In alkaline soils, phosphorus readily reacts with calcium to form sparingly soluble calcium phosphates. The movement of phosphates in soils is very limited, and soils are said to have high "fixing powers" for phosphates. Heavy soils, as a rule, show higher fixing powers than light ones, and soils with high iron contents possess especially strong fixing properties. The two elements mainly responsible for the

fixation of phosphates are calcium, in neutral and alkaline soils, and iron in acid soils (Ross, 2003).

In the last decades, and due to the increasing demand for legume products as well as chickpea, the production was extended towards the alkaline clay soils mainly in the Central Clay Plain of the Sudan where the land is not a limiting factor. Chickpea is also grown in areas other than northern Sudan. Around 1500 to 2500 ha are estimated to be under chickpea along the Rahad river in the central region. Chickpea has a long history of cultivation in this region. The climatic conditions and crop management practices in this area are different from those in the northern region. Summer rainfall is more than 500 mm and minimum temperatures are generally favorable ensuring a longer crop duration as can be seen from the meteorological data of Um Benein Station. Cultivation of chickpea and other crops is spread along both banks of the river Rahad, which is a seasonal river, after the rainy-season water recedes (Ahmed and Bushara 1992).

2.12. Significance of mycorrhiza to enhance phosphorous availability:

Vesicular-arbuscular mycorrhiza (VAM) was found to improve the availability of phosphorous and other immobile elements like zinc and iron (Baylis, 1959). This was thought to increase the root volume through the association with the fungi mycelia. mycorrhizal symbiosis improves nodulation and N₂-fixation in legume crops (Mahdi, 2006).

Mycorrhizal symbioses can increase the spatial availability of P, extending the nutrient absorptive surface by formation of mycorrhizal hyphae. Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of about 74% of angiosperms. In the symbioses, nutrients are transferred by AMF via their extensive mycorrhizal mycelium to plants while in return the fungi receive carbon from the plant. AMF not only influence plant growth through increased uptake of nutrients (e.g. P, zinc, and copper), but may also have nonnutritional effects in terms of stabilization of soil aggregates and alleviation of plant stresses caused by biotic and abiotic factors (Smith and Read, 2008).

A primary benefit of AMF is the improved P uptake conferred on symbiotic plants. In low-P soils mycorrhizal plants usually grow better than nonmycorrhizal plants as a consequence of enhanced direct P uptake of plant roots via the AM pathway.

However, plant growth can be suppressed even though the AM pathway contributes greatly to plant P uptake (Smith and Read, 2008). The growth inhibitions might be caused by the down-regulation of the direct root P-uptake pathway. Recent gene expression study shows that plants induce a common set of mycohrriza-induced genes but there is also variability, indicating that there exists functional diversity in AM symbioses. The differential expression of symbiosis-associated genes among different AM associations is related to the fungal species, plant genotypes, and the environmental factors. Therefore, regulation of direct uptake pathways through epidermis and root hairs and AM pathways requires further investigation (Bucher, 2007).

Any mention of enhancing P and N availability and uptake must make mention of mycohrrizal-plant associations. Mycohrrizal fungi (inclusive of vesicular-arbuscular, ecto-, and ericoid-mycohrrizae) associate with 80% of the terrestrial plant species (Marschner, 1995). The growth of mycohrrizal fungi on and in plant roots dramatically increases the surface area of roots available for soil exploration of nutrients, particularly P, but also N. The uptake of PO_4^- , NO_3^- , and NH_4^+ by external hyphae is a primary contributor to the adaptation of land plants to nutrient-poor soils. Carroll (2001) demonstrated that the diversity of vesicular-arbuscular mycohrrizae in soils was a dominant factor for maintaining biodiversity and ecosystem functioning. Increased mycohrrizal diversity was highly correlated with efficient use of soil P within a mixture of plant species. Legumes, except for a few species, are dependent upon mycohrrizae for efficient P uptake. They note that tree legumes inoculated with rhizobia and mycohrrizae are recommended for reclamation of nutrient-poor, heavily degraded soils. Although the role of mycohrrizae in plant P acquisition has been well documented for more than 30 years, room is available for research progress on their role in acquiring other nutrients (Plassard and Dell, 2010).

Suggested at this time (low nitrogen) to mycohrriza, which thought that it showing the ability of the plant inoculated by mycohrriza on an absorption the nitrogen component from the poor soil in nitrate and thought that the inoculums by this fungi allowed host plant to use ammonium and organic nitrogen existing in the surface level of the soil. The importance of mycohrriza in increasing nutrients absorption efficiency by slow rates because changing in absorption in the root interception resulting more inoculation by mycohrriza with increasing absorption

area and fungi mycelia surfaces. Effect of mycorrhiza on plant growth in the field can differ from nothing to three folds minimally. The improving growth using mycorrhiza more than uninoculated soils. The fungi increasing the growth of many plants and improve the ability of absorption phosphorous from poor soils in phosphorous and able to beneficial exchange with most of the plant minimally change and encourage plant growth. To the legumes showed that improving phosphorous nutrition with (VAM) fungi achieve improving nodules formation and N₂-fixation. Some research used the term infection to describe entering mycorrhiza the plant roots. While other prefer term colonization. It was thought that mycorrhiza enhance the supply of nitrogen to the plant through decomposing the organic materials in the soil released nitrogen. Not only this, even researcher believed that mycorrhiza capable to N₂-fixation (Al-khidir, 2007).

CHAPTER THREE

MATREIALS AND METHODS

The experiment was carried out in winter season in mid November at Food Security Farm of River Nile State at Atbara. Atbara is located at 14°_17°, longitude 33°_55°, and altitude 350m above sea level, within the semi-desert region (Adam, 2002), Appendix 1. The farm soil described as alkaline clay soil. Appendix 2.

3.1. Materials:

3.1.1. Plant materials:

Chickpea seeds (Gram) *Cicerarietinum* l. cultivar Burgig was obtained from Hudiba Research Station, River Nile State.

3.1.2. Rhizobium:

The strain used was *Brady rhizobium* sp. (vigna) strain H12 supplied by Biological N₂-fixation Lab on National Research Center, Khartoum .

3.1.3. Mycohriza:

The strain was used (USDA.3100) supplied by Japan International Co-operation Agency (JICA) Lab, College of Agriculture Studies, Shambat.

3.1.4 Di ammonium Phosphate(DAP fertilizer):

Contains 18% N and 46%P₂O₅.

3.2 Methods:

3.2.1. Treatments:

The experiment treatments were:

1. Control(C).

2. Inoculum(I).
3. Inoculum +Mycohriza (I+M).
4. Inoculum +Mycohriza+ DAP120kg /ha (I+M+DAP120).
5. Inoculum +Mycohriza+ DAP140kg /ha (I+M+ DAP140).

3.2.2. Experiment Design:

The experiment design was used Randomized Complete Block Design (RCBD) with four replicates.

3.2.3. Cultural practices:

3.2.3.1. Land preparation:

The experiment site was ploughed, harrowed, leveled, and ridged and divided into 20 plots. The plot size was 3*4 m².inter-row spacing was 60cm, intera-row spacing 20 cm, and two seeds per hole. Each treatment was repeated four times.

3.2.3.2. Sowing:

3-4 Seeds were sown in holes 20 cm apart on November 2nd, 2014. The plots were irrigated immediately after sowing. Most of the plants emerged 4 days after sowing. Thinning the plants to tow per hole was carried out 7 days after sowing. Prevailing successive irrigations were given at 10_12 days intervals according to the prevailing weather condition. Other cultural practices were carried out according to recommendation.

3.2.3.3. Inoculation:

Both rhizobium and mycohriza inocula were applied just before sowing. The seeds were dipped solution to acquire adhesiveness, and then mixed with inocula till complete coverage with the inoculums.

3.3. Data collection and analysis:

3.3.1. Data collection:

3.3.1.1. Number of Nodules:

Five plants were taken randomly of the side of each plot at mid flowering stage (50%). The nodules were counted, and the mean numbers of nodules per plant were recorded for every plot.

3.3.1.2. Relative Growth Rate (gm/m²/day):

In December 3-4 weeks after sowing, plants from an area 0.5 meter at the middle of each plot were taken randomly and direct in the oven at 700C for two days. The second dry weight was determined 7 days after the first on by the same way, and then the relative growth rate was calculated after (Radford, 1967):-

$$\frac{W_2-W_1}{A*T}$$

W₂=Second dry weight (17th December).

W₁=First dry weight (23th December).

A=Area (Spacing between the ridge (60cm)*0.5m).

T=Time between First and second weight (7 days).

3.3.1.3. Yield and yield components:

3.3.1.3.1. Number of pods per plant:

Counted mean from five plants from an area of 1m² in the middle of the plot at the harvest period in March and number of pods per plant determined.

3.3.1.3.2. Number of seeds per pod:

Counted mean from five plants selected from an area of 1m² in the middle of the plot at the harvest period in March and number of seeds per pod determined.

3.3.1.3.3. 100 seeds weight:

100 seeds from the plant taken from an area of 1m² from each plot then weighted by grams.

3.3.1.3.4. Grain yield t/ha:

Seeds from the plants taken from an area 1m^2 were weighted by grams, and then converted to tons/hectares by the following equation:

$$\text{Grain yield gm/m}^2 * 100$$

3.3.2. Statistical analysis:

The analysis of variance (ANOVA) was carried out on the data collected. The mean of the different treatments were separated used Duncan Multiple Range Test (DMRT) according to little and Hill (1978).

CHAPTER FOUR

RESULTS

4.1. Relative Growth Rate (RGR):

The analysis of variance revealed that there were highly significant differences among All treatments produced of relative growth rate over the control ($P \leq 0.05$) (appendix 3).

The highest values of relative growth rate (7.2750), (6.6250) and (6.3250) were recorded by Inoculum + Mycohriza, Inoculum and Control respectively and the lowest values (2.6500) and (0.7250) were regarded by the Inoculum + Mycohriza + DAP 240kg and Inoculum + Mycohriza + DAP 120kg, respectively (Table, 1).

Table 1: The effect of Inoculum, Mycohriza, DAP120 kg and DAP240kg per ha on Relative Growth Rate:

Treatments	Means of Relative Growth Rate $\text{g.m}^{-2}.\text{day}^{-1}$
C	6.3250 B
I	6.6250 B
I + M	7.2750 A
I + M + DAP 120kg	0.7250 D
I + M + DAP 240kg	2.6500 C
C.V = 5.84%	
Standard Error for Comparison : 0.1949	

The mean followed by the same letters are highly significant different from each other according to (D M R T) at the 0.05 probability level.

4.2. Number of Nodules per plant:

The analysis of variance revealed that there were significant differences among all treatments produced of number of nodules per plant over the control ($P \leq 0.05$) at (appendix 4).

The highest values of number of nodules per plant (2.3500), (1.6000) and (1.3500) were recorded by Inoculum + Mycohriza, Inoculum and Inoculum + Mycohriza + DAP 240kg respectively and the lowest values (0.6500) and (0.5000) were regarded by the Control and Inoculum + Mycohriza + DAP 120kg, respectively (Table, 2).

Table 2: The effect of Inoculum, Mycohriza, DAP120kg and DAP240 kg per ha on Number of Nodules per plant:

Treatments	Means of Number of Nodules
C	0.6500 C
I	1.6000 B
I + M	2.3500 A
I + M + DAP 120kg	0.5000 C
I + M + DAP 240kg	1.3500 B
C.V= 25.20%	
Standard Error for Comparison : 0.2299	

The mean followed by the same letters are significant different from each other according to (D M R T) at the 0.05 probability level.

4.2. Number of Pods per Plant:

The analysis of variance revealed that there were significant differences among all treatments produced of number of pods per plant over the control ($P \leq 0.05$) at (appendix 5).

The highest values of number of pods per plant (38.050), (37.750) and (36.650) were recorded by Inoculum + Mycohriza + DAP 240kg, Control and Inoculum + Mycohriza + DAP 120kg respectively and the lowest values (30.600) and (29.800) were regarded by the Inoculum + Mycohriza + DAP 120kg and Inoculum, respectively (Table, 3).

Table 3: The effect of Inoculum, Mycohriza, DAP120kg and DAP240kg per ha on Number of Pods per Plant:

Treatments	Means of Number of Pods per Plant
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C	37.750 A
I	29.800 C
I +M	30.600 BC
I + M + DAP 120kg	36.650 AB
I + M + DAP 240kg	38.050 A
C.V= 11.50%	
Standard Error for Comparison: 2.8111	

The mean followed by the same letters are significant different from each other according to (D M R T) at the 0.05 probability level.

4.3. Number of Seeds / Pod:

The analysis of variance revealed that there were significant differences among all treatments produced of number number of needs / pod over the control ($P \leq 0.05$) at (appendix 6).

The highest values of number of seeds / pod (1.2550), (1.0850) and (0.9925) were recorded by Inoculum + Mycohriza + DAP 120kg, Inoculum + Mycohriza + DAP 240kg and Inoculum + Mycohriza respectively and the lowest values (0.9225) and (0.9050) were regarded by the InoculumandCotrol, respectively (Table, 4).

Table 4: The effect of Inoculum, Mycohriza, DAP120kg and DAP240kgp per ha on Number of Seeds / Pod:

Treatments	Means of on Number of Seeds / Pod
C	0.9050 B
I	0.9225 B
I +M	0.9925 AB

I + M + DAP 120kg	1.2550 A
I + M + DAP 240kg	1.0850 AB
C.V= 20.50%	
Standard Error for Comparison : 0.1496	

The mean followed by the same letters are significant different from each other according to (D M R T) at the 0.05 probability level.

4.5.100 Seeds Weight (gm):

The analysis of variance revealed that there were significant differences among all treatments produced of 100 seeds weight (gm) over the control ($P \leq 0.05$) at (appendix 7).

The highest values of 100 seeds weight (gm) (18.700), (17.275) and (16.250) were recorded by Inoculum, Inoculum + Mycohrriza + DAP 120kg and Inoculum + Mycohrriza + DAP 240kg respectively and the lowest values (15.050) and (14.950) were regarded by the Inoculum +Mycohrrizaand Cotrol, respectively (Table, 5).

Table 5: The effect of Inoculum, Mycohrriza, DAP120kg and DAP240kgp per ha on 100 Seeds Weight (gm):

Treatments	Means of 100 Seeds Weight (gm):
C	14.950 D
I	18.700 A
I +M	15.050 D
I + M + DAP 120kg	17.275 B
I + M + DAP 240kg	16.250 C
C.V= 3.41%	
Standard Error for Comparison : 0.3967	

The mean followed by the same letters are significant different from each other according to (D M R T) at the 0.05 probability level.

4.6.Grains Yield Tons per ha:

The analysis of variance revealed that there were significant differences among all treatments produced of grains yield tons per ha over the control ($P \leq 0.05$) at (appendix 8).

The highest values of grains yield tons per ha (576.75), (463.50) and (373.50) were recorded by Inoculum +Mycohriza, Inoculum + Mycohriza + DAP 120kg and Inoculum respectively and the lowest values (360.50) and (295.00) were regarded by the Cotroland Inoculum + Mycohriza + DAP 240kg, respectively (Table, 6).

Table 6: The effect of Inoculum, Mycohriza, DAP120kg and DAP240kgp per ha on Grains Yield Tons per ha:

Treatments	Means of Grains Yield Tons per ha
C	360.50 CD
I	373.50 C
I +M	576.75 A
I + M + DAP 120kg	463.50 B
I + M + DAP 240kg	295.00 D
C.V= 12.29%	
Standard Error for Comparison : 35.964	

The mean followed by the same letters are significant different from each other according to (D M R T) at the 0.05 probability level.

CHAPTER FIVE

DISSCUSION

In the this experiment due to the increasing demand for legume products, the production was extended towards the alkaline clay soils mainly in the Central Clay Plain of the Sudan where the land is not a limiting factor. In such soils, the degree of nodulation was monitored to be very poor (Hamid, 2005; Gumaa, 1999; and

Rabih, 1999). They attributed this phenomenon to the impact of the heavy texture of the clay soils mainly to the poor aeration. Elshaikh (1993) showed that the alkaline pH interferes with the availability of some essential nutrient elements e.g. P and Fe to the symbiotic N₂-fixation.

The highest chickpea grain yield t/ha (Table 6) in the study was achieved by the treatment inoculums + mycorrhiza. Inoculation of chickpea seeds with *Bradyrhizobium sp vigna* strain H12 gave the highest number of nodules/ plant (Table 2). Supporting results were reported by several researchers (Saneya, 2012) who agreed that inoculum of many legume seeds with rhizobia is important to increase nodulation and hence N₂-fixation. Research in the Sudan revealed that the inoculums of guar seeds with *Bradyrhizobium* so resulted in 100% increase the dry matter accumulation, number and weight of nodules, and nitrogen and phosphorous content in the plant tissue.

Rhizobium inoculum and nitrogen fertilization significantly increased the shoot and root dry weight of chickpea. This may be attributed to stress during early growth stage. Inoculation increased shoot dry weight after 12 days after sowing in the first season more than 50% in average for the three strains which is comparatively similar to that of nitrogen fertilization (61%). Mahadi (2006) reported that inoculation significantly increased shoot dry weight of chickpea.

This experiment showed poor nodulation, Elshaikh (1993) explained the poor nodulation under the alkaline reaction conditions to its negative effect on the availability of nutrients which are essential to the process. Al Shaikh (1993) also showed that poor nodulation is expected under the alkaline reaction conditions. This may indicate the absence of infective *Rhizobium* strains of chickpea inoculation. This could be supported by the fact that, the study area Atbara has not been cultivated with chickpea. These findings are in agreement with the result of Karrar, (1984) who reported that chickpea was never found to nodulate naturally in Khartoum and Tokar Delta. Also Abd Elsamad (1999) reported that the area in which chickpea is not a traditional crop nodulation was invoked only after nodulation with component *Rhizobium* strain. However, even in areas which chickpea may be grown such as Hudaiba and natural nodulation of the crop was very poor. However, successful nodulation of chickpea in Shambat and in all Al Rawakeeb sandy soil. This may be attributed to the fact that chickpea nodulating

rhizobia high are specific. Chickpea plants also develop a highly specific requirements to their partner rhizobial strain during adaptation to new environments (Ibrahim and Salih, 1980).

The positive interaction of inoculum + mycohriza was only expressed in the relative growth rate (Table 1) and number of nodules (Table 2). Although this effect was not sufficient enough to be allocated in to the end product i.e. the grain yield. This could be attributed to the fact that Chickpea seed is very rich in protein(Al-khidir, 2007). That means the crop demand a very good supply of nitrogen to satisfy good grain yield. This was not the case in this study where the grain yield was not affected significantly (Table 6).In the low grain yield; accordingly, could be explaining by the failure of the nitrogen fixation as mentioned before. The symbiotic nitrogen fixation process was described to take place in four steps. The first step is the root hair infection. The second step is the nodule development. The third step is the nitrogenase synthesis and activity (Rabih et al, 1999). The fourth step took place while the other three steps failed. Such failure; as explained before was due to the poor aeration resulting from the soil heavy texture, and alkaline pH that hindered good availability of essential nutrient. Other factor that could be referred to be the strain which might not be the proper adapted to such condition. Hamid (2005) working in heavy textured alkaline soil at Bakht Elrida found that the inoculum used resulted in very low nodules per plant as compared to those produced by the control treatment.

Applying *Glomus fasciculatum* inoculums (VAM) improve the availability of phosphorous to the guar plants grown on Shambat alkaline clay soil in the experiment. Baylis (1959) was the first to suggest that VAM increase the availability of phosphorous and the other immobile nutrient elements like iron and zinc. Al-Shiekh (1993) stated that phosphorous plays significant role in the process of N₂-fixation, mainly in the achievement of the bacteria to the root hairs as well as energy needed for the synthesis of the nitrogenase enzyme as enhancing iron availability through mycohrizal symbiosis leads also to improve N₂- fixation, because iron is very essential nutrient element in the synthesis of the nitrogenase enzyme.

Dual inoculation of soybean with both *Bradyrhizobium japonicum* and Vesicular Arbuscular Mycorrhiza (*Glomus* sp) fungi synergistically enhanced nodulation, dry matter, nitrogen and phosphorous content (Atabani, 1988).

The study resulted statistically significant differences between all treatments in primary morphological yield components (Table 3, 4, and 5). It is natural and that the number of pods per plant is directly correlated with the grain yield in all legume crops. The character reflected the same trend in the study i.e. where there was no increase in the grain yield since there was no increase in the number of pods per plant. This could be attributed to the failure of the N₂-fixation process in this study.

Nitrogen is one of the most essential elements needed for the plant growth. It is found in many forms which urea is the second most popular carrier. When nitrogen status was low, 90% increase in seed yield of chickpea was obtained with 43 kg N/ ha, while 170% increase was obtained with 84 kg N/ ha (Abd Elsamad, 1999).

Inoculation and nitrogen fertilization supplied plants with their nitrogen requirement, this led to an increase in nodule number and weight consequently causing a vigorous plant with the ability to support more pods. Gumaa (1999) reported that nodulation of chickpea pod number and pod dry weight significantly. Seeds per pod and weight of 100 seeds, since they are genotypic in nature, usually do not respond well to change in environment is the number of pods per plant. Such a trend is natural and often in all legume crops. This was supported by several previous studies e.g. Gumaa (1999) and Hamid (2005) in cluster bean.

The heavy texture of the clay soil at the Food Security Farm affected the nodulation positively. Similar results of poor nodulation under the conditions of heavy textured alkaline soils were reported by (Gumaa, 1999; Rabih, 1999; and Hamid, 2005). Hamid (2005) explained the poor nodulation under alkaline reaction conditions to its negative effect on the availability of nutrients like iron and molybdenum which are essential to the process. This was attributed to the poor aeration and water retention. Another factor might be referred to the use of unsuitable strains. The effect of the land history was reported by Hamid (1981). The suitability and adaptability were not very clear in the efficiency of the native strain inoculated chickpea.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1 Summary:

To evaluate the effect of Rhizobium, Mycorrhiza, Inoculum + Mycorrhiza, Inoculum +Mycorrhiza+DAP 120kg and Inoculum +Mycorrhiza+DAP 240kg on the Nodulation, Growth and Yield of Chickpea a one season field experiment was carried out on the Food Security Farm at the River Nile State in the winter season of 2014. A randomized complete block design with four replicates was used for this purpose.

There were significant differences between all treatments on the Nodulation, Growth and Yield of Chickpea.

6.2 Conclusion:

Based on the results obtained in this study the following conclusion could be drawn:

1. Inoculation with another specific *Bradyrhizobium sp.*
2. Inoculation with *Glomus fasciculatum*.
3. Application of 120kg per hectare (Diammonium phosphate) at planting (Contains 18% N and 46% P₂O₅) should be made in order to increase yield.
4. The experiment should be repeated in another season to confirm the results.
5. Further studies with different combinations were needed to support the hypothesis.

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Appendices

Appendix1:The semi– desert climate:

Sun- shine duration	3650 hour/year
Solar radiation	22.7 MJ / m ² /day
Maximum temperature	42 c° (May)
Minimum temperature	12c° (January)

Temperature range	30c°
Rainfall	100-250 mm/annum
Evaporation	2400 mm/ annum

Appendix 2: Chemical and physical properties of the field soil:

PH	8.0
ECC ds/m	1.7
SAR	6
Soluble cation (meq/1)	
Ca+Mg	0.9
Na	1.0
K	0.2
CL meq/L	1.8
N%	0.08
P p.p.m	7
CaCo ₃ %	2.00
Sand %	37
Silt %	15
Clay %	48

Appendix 3: ANALYSIS OF VARIANCE:

3.1. Relative Growth Rate:

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	0.308	0.1027	
Replications	4	131.912	32.9780	433.92
Errors	12	0.912	0.0760	

Total	19	133.132		
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Coefficient of variation:5.84 %

3.2. Number of Nodules:

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	0.4420	0.14733	
Replications	4	9.0280	2.25700	21.36
Errors	12	1.2680	0.10567	
Total	19	10.7380		

Coefficient of variation: 25.20%

3.3. Number of Pods per Plant:

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	47.238	15.7460	
Replications	4	260.252	65.0630	4.12
Errors	12	189.652	15.8043	
Total	19	497.142		

Coefficient of variation: 11.50%

3.4. Number of Seeds / Pod:

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	0.07320	0.02440	
Replications	4	0.32887	0.08222	1.84
Errors	12	0.53705	0.04475	
Total	19	0.93912		

Coefficient of variation: 20.50 %

3.5.100 Seeds Weight (gm):

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	2.8015	0.93383	
Replications	4	39.9720	9.99300	31.76
Errors	12	3.7760	0.31467	
Total	19	46.5495		

Coefficient of variation: 3.41 %

3.6.Grains Yield Tons per ha:

Source	Degree of freedom	Sum of square	Mean square	F value
Treatments	3	1411	470.2	
Replications	4	190405	47601.2	18.40
Errors	12	31041	2586.8	
Tal	19	222857		

Coefficient of variation: 12.29 %