RESPONSE OF CHICKPEA (C*icer aritienum* L.) TO SULPHUR AND ZINC NUTRIENTS APPLICATION AND *RHIZOBIUM* INOCULATION IN NORTH WESTERN ETHIOPIA

MSc. THESIS

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JULY 2017
HARAMAYA UNIVERSITY, HARAMAYA

Response of Chickpea (*Cicer aritienum* L.) to Sulphur and Zinc Nutrients Applications and *Rhizobium* Inoculation in North Western Ethiopia

A Thesis Submitted to School of Natural Resource Management and Environmental Science, Post Graduate Program Directorate
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN AGRICULTURE (SOIL SCIENCE)

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July 2017
Haramaya University, Haramaya

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this Thesis entitled **"Response of Chickpea (C*icer aritienum* L.) to Sulphur and Zinc Nutrients Applications and *Rhizobium* Inoculation in North Western Ethiopia"** prepared under our guidance by **Beza Shewangizaw Woldearegay**. We recommend that it be submitted as fulfilling the Thesis requirements

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As members of the Board of Examiners of the MSc. Thesis Open Defense Examination, we certify that we have read and valuated the Thesis prepared by **Beza Shewangizaw Woldearegay** and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Agriculture (Soil Science)

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BIOGRAPHICAL SKETCH

The author was born on October 17, 1984 to Mr. Shewangizaw Woldearegay and Mrs. Bizunesh Agonafer at Inewari, North Shewa Zone, Ethiopia. He attended his elementary and secondary schools education in Inewari Elementary school and Inewari high school, respectively. Following the successful completion of his secondary education, He joined Mertule Mariam Agrcultural, Technical, Vocational, Education Traning (ATVET) College in October 2002 and graduated with Diploma in Natural resource management in July 2004.

After his graduation, he was employed as Natural resource expert in the Ministry of Agriculture at the District Development Office of Mortina Jiru Woreda since August 2004. After serving for one year and six months, he joined Debra Birhan Agricultural Research Center as a technical assistance in February 2006. After serving the center as technical assistance for two years and six month he joined Bahir Dar University in September 2008 and graduated with BSc degree in Natural Resource Management in 2011. From 2011 until he joined the Post Graduate Directorate Program of Haramaya University for his master degree in soil science in September 2015, he served as a soil fertility researcher and soil and water management research directorate coordinator in Debre Birhan Agricultural Research Center of Amhara Agricultural Research Institute (ARARI).

ACKNOWLEDGEMENTS

My special thanks goes to my major advisor Mr. Anteneh Argaw, for his commitment and dedication in supervising me throughout my research work. His continuous awareness and encouragement over the whole period of my study was crucial to the completion of this thesis. I benefit tremendously from many of his wise suggestions on doing research, presenting papers, searching scientific publications. The example of excellence that his research work published in international journal and presented in Haramaya University always incentivized me to do my best work. Moreover, his nice treatment makes me always surprising.

My appreciation also goes to my co-advisors Dr. Tesfaye Feyisa and Dr. Endalkachew W/Meskel. Their keen eye for details and their pointed questions have steered me in the right direction at every turn. In spite of their busy schedule and overwhelming responsibilities, they always extended full attention, patient hearing and complete support to me, without which the present work could not have come to light.

I'd like to express my gratitude to Amhara Agricultural Research Institute and International Livestock Research Institute/Putting Nitrogen working to African Small holder farmers (ILRI/N2Africa) project for their financial support.

My heart felt gratitude and appreciation is also extended to Abiy Legesse, Tsegaye Getachew, and Abiro Tigabiye for their kind and generous supports of finance, vehicle and other materials, without which this work would have not been materialized.

This MSc. Thesis would have not been possible without the guidance and support of many exceptional scholars in Haramaya University, Amhara Agricultural Research Institute, International Livestock Research Institute and the great support of my friend, family and local farmers. I would like to take this opportunity to express my sincere gratitude to them, though a few words cannot adequately capture the magnitude of my appreciation.

I gratefully acknowledge the support from all the staff of Debrebirhan Agricultural Research Center and Haramaya University, Tesgaye Getachew, Belhu Nigatu, Zebideru Mekonen, Ayele Desalegn, Genet Taye, Biruk, Kenzemed Kassie, Temesgen, Getachew Lemma.

My thanks also goes to Lisanu Getaneh for his sincere and interesting support during the field work and laboratory analysis and I am also very grateful to Girma Moges (driver in Debrebirhan Agricultural Research Center).

The author would also like to acknowledge ILRI/N2A staff members; Birhan Abdulkadir, Simret Yemane and Tamiru Amanu. Especially Birhan is well acknowledged for his nice treatment and valuable comments starting from the draft of the proposal and implementation of the plan. I would be negligent if I didn’t mention the farmers at the experimental sites and their families during my stay in Tsion and Denzaz Kebele. Here, I would like to thank Mihretu Bitew, Tilahun Nega, Zena Mihretu, Tatu Mihretu, Negist Mihretu, Yeshiwas Tilahun, Getnet Tilahun, and Antehun for their extreme generosity in sharing their indigenous/local knowledge on the field work, their sincerity in helping me undertaking the field work and serving me during my stay in Tsion and Denzaz.

I would also like to express my sincere gratitude to the following persons who support me in many different ways during the preparation and completion of the study: Mr. Daniel Admasu, Baye Ayalew, Ayalew Addis, Hailu Kindai, Muuz Gebretsadik, Solomon, Yonas Worku, Semachew, and other members and researchers of Gonder Agricultural Research Center.

Last but certainly not least, I want to thank my wife Miraf Zenebe, for her unconditional support and huge sacrifices, she has always been supportive to my academic pursuit and for her endurance during the time I had been away from home. I appreciate it was hard time but I had no alternative, sorry for that. I would also like to thank my lovely babies (Fikir Beza and Maryamawit Beza), as their innocent and heart melting smiles makes my MSc. research life much happier. Finally, my parents, my lovely sister and brother (Birhan and Tringo) deserve special thanks as they gave me great financial and moral support.

LIST OF ACRONYMS AND ABBREVIATIONS

ADP	Adenosine Diphosphate
ANOVA	Analyses of Variance
ANRS	Amhara National Regional State
ARARI	Amhara Regional Agricultural Research Center
ATP	Adenosine Triphosphate
BNF	Biological Nitrogen Fixation
CIMMYT	International Maize and Wheat Improvement Center
CSA	Central Statistical Agency
DTPA	Dietylene Trimaine Penta Acetic Acid
ESS	Ethiopian Soil Science Society
ESS	Ethiopian Soil Science Society
EthioSIS	Ethiopian Soil Information System
FAO	Food and Agricultural Organization
GARC	Gondar Agricultural Research Center
GOZOARD	Gondar Zuria Office of Agriculture and Rural Development
HI	Harvest Index
LSD	Least Significance Difference
m.a.s.l.	Meters Above Sea Level
MBI	Menagesha Biotechnology Industry
MoARD	Ministry of Agriculture and Rural Development
MPN	Most Probable Number
NifTAL	Nitrogen fixation by Tropical Agricultural Legumes
OC	Organic Carbon
OM	Organic Matter
PHI	Phosphorus Harvest Index
PSB	Phosphorus Solubilising Biofertilizers
PUE	Phosphorus Use Efficiency
RNA	Ribonucleic Acid
TSP	Triple Super Phosphate

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ABSTRACT

In sub-Saharan Africa, plant nutrient deficiency, due to nutrient mining, is a major growth limiting factor for crop production. As a result, some soils become non responsive to Rhizobial *inoculation. In an effort to find out possible correction, a field experiment was carried out on-farm, during 2016/17 growing season, at Gondar Zuria woreda in Tsion and Denzaz Kebeles to evaluate the effect of Rhizobium inoculation, S and Zn application on yield and yield parameters, nodulation, N and P uptake on chickpea. The soil at the experimental sites were previously diagnosed as non responsive to Rhizobium inoculation and P fertilizer application on chickpea* (Cicer aritienum *L.*) *and had low OM, N, P, S and Zn. The experiment included twelve* treatments *developed via factorial combination of two level of inoculation (*Rhizobium *inoculated, un-inoculated), three level of S (0, 15, 30 kg Sulphur ha*$^{-1}$*) and two levels of Zn (0, 1.5 kg Zinc ha*$^{-1}$*). The treatment was laid out in randomized complete block design with three replications. All plots have received basal application of 20 kg N ha*$^{-1}$*and 20 kg P ha*$^{-1}$ *uniformly. Zinc in the form of* $ZnSO_4$ *was applied through foliar application and the remaining nutrients were applied at planting directly to the soil. Mean separation was made using the least significant difference (LSD) test at 5% level of probability. Analysis of variance showed that except plant height at both location and shoot dry weight and number of seed per pod at Tsion, all the remaining growth parameters (root length and root dry weight), yield and yield related traits (number of primary branches, number of pod, number of seed, hundred seed weight, grain and straw yield), crop phenology (days to 50% flowering and days to physiological maturity), nodulation scores (nodule number, nodule volume, nodule dry weight, effective nodule and nodulation rating), N and P uptake at both locations were significantly affected by the treatments. The highest (1775.5 kg ha*$^{-1}$*) mean value of seed yield over locations was obtained from combined application of* Rhizobium *and 30 kg S ha*$^{-1}$ *which resulted in 28 % (389 kg ha*$^{-1}$*) increase over the control. The result also indicated that P use efficiency of chickpea was improved with* Rhizobium *inoculation and S fertilizer application. The partial budget analysis also showed that the maximum (ETB 37069 ha*$^{-1}$*) and minimum (ETB 30050 ha*$^{-1}$*) net benefit were obtained from combined application of* Rhzobium *inoculation and 30 kg S ha*$^{-1}$*and from the control check, respectively. The result determined a net benefit penalty of 23.4% (ETB 7019 ha*$^{-1}$*). Hence,* Rhizobium *inoculation with application of 30 kg S ha*$^{-1}$ *could be recommended for chickpea production at the experimental locations in Gonder Zuria Woreda.*

Kee word*:* Rhizobium *inoculation, growth parameter, nodulation, yiled related trait, P uptake.*

1. INTRODUCTION

It is a plain fact that nitrogen (N) is the key component of protein for human and animal consumption and it required for all plants for growth and development (Adler, 2008). It is the structural component of protein and nucleic acid. Nitrogen is also essential for synthesis of chlorophyll which is essential for capturing energy from sun light during photosynthesis (Grham and Vance, 2000; Dordas and Sioulas, 2008; Waraich *et al*., 2011). Crop yield can be increased by maintaining soil fertility and use of sufficient and balanced plant nutrients. Therefore, adequate supply of N is necessary to achieve potential yield.

Nitrogen deficiency is a major factor limiting crop production all over the world (Salvagiotti, *et al*., 2008; Aminifard *et al*., 2010). This constraint is also common in the tropics and subtropics (Endalkachew, 2011). However, Bagayoko *et al*. (2011) reported that the use of fertilizers by African farmers was limited due to poor accessibility, availability and high prices. According to Yifru *et al*. (2007), chemical fertilizer played role in agriculture but the current increasing price and application below the recommended rate are the main limiting factors for most Ethiopian farmers for better production of crops. Hence, interest towards environmentally friendly sustainable agriculture practice, organic farming system has been growing **(**Rigby and Caceres, 2001; Lee and Song, 2007). Therefore, there is an urgent need to realize a vital and cheaper source of fertilizer having eco-friendly approach.

Pulse are leguminous crops which are rich in protein, vitamins and other nutrients are extensively cultivated for human consumption. These crops have the ability to reduce atmospheric N_2 to usable form through biological nitrogen fixation (BNF) in association with root nodule bacteria. Legumes have special bacteria in their root system and make use of N from the air (Adjei *et al*., 2001). This association contributes 50-70 million tons annually to the global agricultural N budjet (Unkovich *et al*, 2008), this account for 40 to 70% of total global nitrogen input (Kahindi and Karanja, 2009). The major root nodule bacteria associated with pulse crops are; *Rhizobium*, *Bradyrhizobium, Mesorhizobium*, and *Azorhizobium,* collectively called rhizobia, can infect plants, leading to symbiotic interaction resulting in root nodule formation.

With these nodules, bacteria live in differentiated form, the bacteroid, and fix nitrogen by reducing atmospheric nitrogen to ammonia (Adjei *et al*., 2001).

The formation of an effective symbiosis requires the existence of specific rhizobia in the soil that can nodulate host legume or inoculation of with effective rhizobia, and suitable environmental factors (Choudhry, 2012).

The major the abiotic factors that affect effective symbiosis are; nutrient, pH, temperature, water holding capacity, water stress, salinity and nitrogen level are the major factors affecting BNF (Keerio, 2001; Panchali, 2011).

For several years now, different studies have been undertaken on inoculation trial of several pulse crops in Ethiopia (Desta, 1988; Angaw and Asfaw, 2006). Accordingly, these field trial showed positive response of faba bean (*Vicia faba* L.) and chickpea (*Cicer arientinum* L.) to inoculation and NP fertilizer application. They also showed that inoculation increased the productivity of different pulse crops in some parts of Ethiopia. Several authors also indicated the positive effect of *Rhizobium* inoculation alone and in combination with NP fertilizer on different soil types (Asegilil, 2000; Amanuel *et al*., 2000; Ayneabeba *et al*., 2001). However, the productivity of chickpea in Ethiopia when compared to the potential yield is still very low. For instance, the national average productivity of chickpea (1.89 tone ha^{-1}) (CSA, 2015) was still lower than 3.2 tone ha^{-1} which was recorded in Newzland (Verghis, 1996) and very lower than its potential yield (5.5 tone ha^{-1}) obtained on experimental stations in Ethiopia (Belay, 2006). This wide yield gap clearly indicates that research on chickpea should look beyond breeding and selection of improved varieties for yield and disease resistance.

According to O'hara *et al.* (1988) to ensure full benefit from nitrogen fixation by legume symbiosis, successful breeding and management strategies should consider the whole legume-*Rhizobium* system and selection of improved legume symbiosis. Around the world, different evidences revealed that inoculation of legumes with effective *Rhizobium* can increase the yield and the nitrogen fixing capacity (Beck and Duc, 1991; Ben Romdhane *et al*., 2008; KÖpke and Nemecek, 2010). On the other hand, due to environmental constraints and effectiveness of the native rhizobia, lack of response to inoculation in field experiments have been frequently reported worldwide and raising doubts about the usefulness of inoculation (Graham, 1981; Buttery *et al*., 1987; Hameed *et al*., 2004).

Several works indicated that *Rhizobium* inoculation integrated with the application of S, Zn, and other plant nutrients improved pulse crops production compared to *Rhizobium* inoculation alone *(*Togay *et al*., 2008; Bahure *et al*., 2016, Valenciano *et al*., 2011). According to Ganeshamurthy and Reddy (2000), an adequate supply of mineral nutrients to legumes enhances nitrogen (N) fixation and yield. This is due to the role of those nutrients in both plant growth and the symbiosis between rhizobia and the host plant. S affects leguminous plant species growth through its effect on N_2 fixation process and involvement in the process of nitrogen fixation. Because of the relatively high S content of nitrogenase (Mortensen and Thornley, 1979) and ferredoxin (Yoch, 1979), S deficiency may affect N_2 fixation. Ferredoxin has a significant role in nitrogen dioxide and sulphate reduction, the assimilation of N by root nodule bacteria and free living N-fixing soil bacteria (Scherer *et al*., 2008). Moreover, legumnious plant reqire a large quantity of S because of their high protein content. Zinc is also involved in various host plant metabolic processes, nodule growth and N_2 fixation process. Zinc has also important role in activating plant enzymatic system, synthesis of chlorophyll and carbohydrates.

In Ethiopia, Habtegebrial and Singh (2006) found positive effect of P application in nodulation and N_2 fixation of faba bean. Beside this, *Rhizobium* inoculation plus P application increased the nodulation and N_2 fixation of faba bean (Amanuel and Tanner, 1991; Habtegebrial *et al*., 2007). Starter N application also increased the yield and nodulation of common bean in eastern part of Ethiopia (Anteneh and Daniel, 2016; Daba and Haile, 2000). In contrast to these findings, the effect of P application and *Rhizobium* inoculation on chickpea have been variable (unpulished data). There was non-significant effect of P application and *Rhizobium* inoculation on chickpea at Gondar Zuria Woreda (Dinzaz, Degolla and Tsion Kebeles) (Unpublished data). However, O'hara *et al*. (1988) found that correction of deficient nutrients (S and Zn) on top of P significantly improved the effectiveness of *Rhizobial* inoculation in terms of nodule development, nodule functioning and assimilation of nitrogen by the host plant.

One way of improving the low productivity of chickpea is combined application of efficient, competitive and persistent strains of *Rhizobium* with deficient nutrients (P, S and Zn). To attain this, it is essential to generate information by studying the response of chickpea to combined application of *Rhizobium* inoculation and S and Zn nutrient application.

Hence, we hypothesized that correcting deficient nutrients in the study site improves the effect of P application and *Rhizobium* inoculation on nodulation and productivity of chickpea. This experiment was therefore, initiated to evaluate the effect of correcting limiting plant nutrient in effectiveness of *Rhizobium* inoculation in chickpea in selected sites of north western Ethiopia.

The specific objectives of the study were

- To evaluate the effect of combined application of *Rhizobium* inoculant, S and Zn nutrients on nodulation and yield of chickpea (*Cicer arientinum L.*)
- To evaluate the effect of inoculation, S and Zn nutrient application on P use efficiency (Uptake) of chickpea in the study sites.

2. LITERATURE REVIEW

2.1. Biological Nitrogen Fixation (BNF)

Though molecular nitrogen represents nearly 80% of the earth's atmosphere, it is chemically inert and cannot be directly assimilated by plants. Only limited numbers of prokaryotes are able to convert the N_2 molecule into a usable form of N through a process known as biological nitrogen fixation (Allito *et al*., 2015). Biological nitrogen fixation is the process that changes inert N_2 that is abundant in the atmosphere to biologically useful NH_3 naturally by the help of prokaryotic organism such as eubacteria and cyanobacteria (Giller, 2001). Other plants benefit from nitrogen fixing bacteria when the bacteria die and release N to the environment, or when the bacteria live in close association with the plant. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules.

Biological nitrogen fixation is highly energy consuming process. Nitrogen molecules is reduced to NH_3 under consumption of ATP and redox equivalents, and is associated with the formation of H_2 as a byproduct ($N_2 + 8\ H+1 + 8\ e- +16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$) (Lodwig and Poole, 2003). The enzyme that catalyzes the reaction is called nitrogenase and consists of the dinitrogenase reductase protein (Fe protein) and dinitrogenase (MoFe protein) which actually catalyzes the reduction of N_2.

In terrestrial ecosystem, there are three major strategies to fix or reduce atmospheric nitrogen to plant usable form: symbiotic, non-symbiotic or associative, and free livings N_2 fixation. The most important N_2 fixing agents in agricultural systems are the symbiotic associations between legumes and the microsymbiont rhizobia bacteria (Giller, 2001) followed by non-symbiotic nitrogen fixation. Since free-living diazorophs are heterotrophic bacteria and are subjected to substrate limitation and their contribution in nitrogen fixation is very small (Marschner, 1995). The terrestrial input (natural origin and human activities) of N from BNF accounts for approximately 240–280 t N $year^{-1}$ (Galloway, 1998), this amount is much higher compared to the 85 t N $year^{-1}$ consumed as nitrogenous fertilizers all over the world in 2002 (FAO, 2008).
The nitrogen-fixing symbiosis between legumes and prokaryotic microorganism (bacteria) is characterized by the formation of nodules, which are subsequently colonized by the specific microsymbionants. The prokaryotic partners include number of family *Rhizobiaceae*,

collectively named rhizobia (genera *Bradyrhizobium, Rhizobium, Mesorhizobium, Ensifer*, or *Sinorhizobium, Azorhizobium, Allorhizobium*) as well as other taxa (*Burkholderia* (Moulin *et al*., 2001), *Ralstonia* (Chen *et al*., 2001), *Methylobacterium* (Sy *et al*., 2001), and *Devosia* (Rivas *et al*., 2002).

The first step in symbiotic interaction is infection by the microsmbionant. Infection by the microsymbionant may occur on developing root hairs, at the junction of lateral root or the base of the stem. When nitrogen in the soil is insufficient, legumes release flavonoids which signal to rhizobia that the plant is seeking symbiotic bacteria (Ndakidemi and Dakora, 2003). In response, the rhizobia releases nodulation factor which stimulates the plant to create deformed root hairs (Banfalvi and Kondorosi, 1989). Rhizobia then form an infection thread for allowing them to enter the root cells through root hairs (Gage *et al.,* 1996). When the rhizobia are inside the root cells, the cells divide rapidly to form nodule (Dudley *et al*., 1987). Then the rhizobia convert atmospheric nitrogen into ammonia, a form that is directly used by the plant for synthesis of amino acids and nucleotides, the plant provides the bacteria with sugars, hence the symbiosis is established.

The transformation of the bacteroids is accompanied by the synthesis of hemoglobin, nitrogenase and other enzymes required for N_2 fixation (Rolfe and Gresshoff, 1988). The plant must contribute a significant amount of energy in the form of photosynthate (photosynthesis derived sugars) and other nutritional factors for the bacteria. The bacteria in turn supply the plant with ammonium or ammonia.

Depending on the legume species and germination condition, small nodules are visible with naked eye within a week after infection. In the symbiotic nitrogen-fixing organisms such as *Rhizobium*, the root nodules can contain oxygen-scavenging molecules such as leghemoglobin, which shows as a pink color when the active nitrogen-fixing nodules of legume roots are cut open. Leghemoglobin may regulate the supply of oxygen to the nodule tissues in the same way hemoglobin regulates the supply of oxygen to mammalian tissues. When nodules are young and not yet fixing nitrogen, they are usually white or grey inside (Nair, 2007).

2.2. Effect of *Rhizobium* Inoculation on Pulse Production

According to Ladha *et al*. (1988), the use of mineral N fertilizer in production has increased but its agronomic use efficiency is less than 50%. But unfortunately a substantial amount of the urea-N is lost through different mechanisms causing environmental pollution problems (Chowdhury and Kennedy, 2004). Rigby and Caceres (2001) reported that the extensive use of chemical fertilizer in agriculture is resulted in environmental health problem and do have a negative impact on consumers' health. As a result interests have been increasing in environmentally friendly sustainable agriculture practice and organic farming system. Therefore biofertiliztion is of great importance in alleviating environmental pollution and deterioration of nature (Elkoca *et al*., 2008). Vessey (2003), indicated that biofertilizers are materials with beneficial inoculants, when applied to the soil, seed or plant surface colonizes the rhizosphere or the interior of the plant and promote growth by facilitating the supply or availability of nutrient through natural processes like nitrogen fixation and phosphate solubilizing. The different types of biofertilizers includes: nitrogen fixing biofertilizers, e.g. *Rhizobium, Bradyrhizobium, Azospirillum* and *Azotobacter;* phosphorus solubilising biofertilizers (PSB), e.g. *Bacillus, Pseudomonas* and *Aspergillus*; phosphate mobilizing biofertilizer, e.g. *Mycorrhiza*; and plant growth promoting biofertilizers, e.g. *Pseudomonas*. Increasing and extending the role of *Rhizobium* biofertilizers can reduce the need for chemical fertilizers and decrease adverse environmental effects (Erman *et al*., 2011; Namvar *et al*., 2013).

According to Namvar *et al*. (2013), legume is a major contributor to sustainable agriculture through its ability to fix N_2 to usable form and as a rotation crop that is important for diversification of agricultural production systems. As a result the ability of legume to fix atmospheric N and their residual impact on soil N status makes its rotation in agricultural system important (Glasener *et al*., 2002). According to Giller *et al*. (1998) the quantity of N that the legume fixed and the N which is incorporated in to the soil and the time-span of the decomposition of the residue and the synchrony with nutrient need of the subsequent crops are factors that affect to what extent the legume crop can benefit a subsequent crop.

According to KÖpke and Nemecek (2010), under broad spectrum condition faba bean can symbiotically fix atmospheric nitrogen and make available for the next crop. So inoculation of faba bean with effective rhizobia increases nodulation, N fixation, growth and yield of their host plant (Beck and Duc, 1991). Walley *et al.* (2007) also reported that effective inoculation of legume can fix sufficient quantities of N. Therefore, inoculation with efficient rhizobia is recommended in environments where compatible rhizobia are absent, soil rhizobial population density has been reduced, or where rhizobia are shown to be less effective (Chemining'wa and Vessey, 2006). But in some cases, resident soil rhizobia including native rhizobia and those naturalized through past inoculation, may have impacting inoculation success through its impact on competance for nodule occupancy with introduced rhizobial strains (Denton *et al*., 2002).

Different researches indicated that amount of N fixed by faba bean vary greatly, Duc *et al*. (1988) reported that it can fix 40 Kg ha^{-1}. But according to Danso (1992) faba bean can fix about 120 Kg ha^{-1}. Between these values, Brunner and Zapata (1984) reported that faba bean can fix about 93 Kg ha^{-1}. Fassil (2010) reported that growing faba bean can improve the orginal soil N by 10.6 times than the orginal soil N.

The effect of *Rhizobium* inoculation on chickpea yield depends on the native rhizobial status. If previously well nodulated chickpea was grown, *Rhizobium* inoculation is not required. Rhinhart *et al*. (2003) reported that chickpea fix 60-80% of its nitrogen requirement with symbiotic association with the nitrogen fixing bacteria, N application is not necessary for the crop. This can be achieved only if the bacteria is present in the soil. According to ICRISAT (1987), inoculation with *Rhizobium* is required where chickpea is being grown after paddy or chickpea is being introduced for the first time. According to Ben Romdhane *et al*. (2008) inoculating chickpea with competitive strain of rhizobia can improve the growth and yield of chickpea and hence this is one of the most important and economically feasible way of increasing productivity of the crop. Seed inoculation of chickpea can improve grain yield and quality up to 50 percent (Kyei-Boahen *et al*., 2002). Fatima *et al*. (2008) also reported that *Rhizobium* inoculation increase plant height, grain yield and nitrogen fixation in chickpea. Togay *et al*. (2008) found that inoculation with *Rhizobium* significantly increased the plant height, number of branches, pods and seeds per plant, grain and dry matter yield in chickpea.

Researches conducted in Oromia, Amhara (Gondar) and South Nations, Nationalities and people region indicated chickpea respond strongly to inoculation (Ali *et al*., 2004). Studies in other countries also indicate that chickpea positively responds to *Rhizobium* inoculation. In Iran and

Canada, the grain yield was found increased by 8 to 40%. In Pakistan, combined application of *Rhizobium* with 90 kg P 90 kg ha^{-1} increse grain and stover yield of chick pea from1600 to 3100 and from 4350 to 7500 kg ha^{-1}, respectively (Ali *et al.*, 2004). Other finding also reported that inoculation with *Rhizobium* and mycorrhiza improved both grain and stover yields by about 60% in Turkey (Erman *et al.*, 2011)

2.3. Factors Affecting Biological Nitrogen Fixation

Nitrogen fixation is one of the important soil microbial activities affected by all on-going processes in soil as well as other microorganisms. The many different environmental factors in soil affecting these processes are low or extremely high level of soil moisture, salinity, deficiency of nutrient, extreme temperature, water holding capacity, nitrogen level, unfavorable soil pH, mineral toxicity (Giller, 2001 and Panchali, 2011). Many of these factors affect many aspect of nitrogen fixation and assimilation, as well as factors such as respiratory activities, gaseous diffusion and the solubility of dissolved gasses, which ultimately affect host plant-*Rhizobium* association and hence plant growth (Keerio, 2001).

The moisture stress can adversely affect the nodule functions. The drought conditions can reduce nodule weight and nitrogenase activity. According to Ramos *et al*. (2003) after exposure to the moisture stress for 10 days, the nodule cell wall starts to degrade resulting in senescence of bacteroids. Durrant (2001) also found the direct and indirect effect of moisture to nitrogen fixation. Low moisture condition in soil resulted in a hindrance to nodule respiration as a result nitrogen in nodule moves out slowly. Several studies conducted in Egypt and abroad have shown that nodulated plants of faba bean exhibit a high degree of correlation between N_2 fixation and soil water content (Abdel-Ghaffar, 2009).

High salt level can directly affect the early infection between the *Rhizobium* and legume in nodule formation (Singleton and Bohlool, 1984). According to Caesar and Rusitzka (1982) as cited by Abdel-Ghaffar (2009) this harmful effect is attributed to; direct toxicity of the salt, reduction in availability of soil water due to high osmotic pressure of soil solution, changes in availability of nutrients due to ion antagonism, and changes in physical properties of soil restricting water movement or reducing root penetration.

Agricultural management factors can also influence BNF. Choice of variety, plant density and inoculation also affect BNF and hence plant growth and development (Ronner and Franke, 2012). According to Solomon *et al*. (2012) legume species and variety can have an effect on the amount of nitrogen fixed. Disease conditions which affect the plant growth and development can also affect the persistent *Rhizobium* strain to perform root infection and ultimately the ability of the legume to fix atmospheric N to its full capacity (Panchali, 2011). Higher plant population density shows either positive or negative for percentage of nitrogen fixed from the atmosphere. Higher density may increase the amount of fixed nitrogen due to increased competition for soil nitrogen. On the contrary higher density may have a negative impact on nitrogen fixation as a result of competition for other nutrient and moisture (Naab *et al*., 2009; Makoi *et al*., 2009). Tillage practice, selection of effective and responsive crops, appropriate cropping system, method and time of sowing, use of agrochemicals, use of *Rhizobium* culture and its frequency, the way of handling the inoculant and the method of inoculation also affect BNF by affecting both the crop and the microbial activity (Kantar *et al*., 2010).

According to Abdel-Ghaffar (2009) failure of faba bean to respond to inoculation could be attributed to the presence of an abundant supply of effective *R. leguminosarum* strains in soil, inefficient inoculant caused by non-viable cell, contaminated with antagonistic organisms, unsuited for the host plant or low in density of *Rhizobium* cells, direct contact of inoculated seeds with fertilizers, toxic chemicals, lack of certain nutrient such as P, Mo, Zn, Co, B, and/or (g) excess P or N fertilization. O'hara *et al*. (1988) reported that multiplication of rhizobia, nodule initiation, nodule development, nodule functioning and assimilation of nitrogen by the host plant is negatively affected by Ca, Co, B/Fe, Mo and Zn deficiency.

2.4. Essential Mineral Nutrients for BNF

The essential mineral nutrients for symbiotic legume nitrogen fixation are those required for the normal establishment and functioning of the symbiosis. Based on Arnon and Stout (1939) the following chemical elements are known to be essential for the legume- *Rhizobium* symbiosis: C, H, O, N, P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, Mo, B, C1, Ni and Co. Each essential nutrient has specific physiological and biochemical roles and there are minimal nutrient concentrations required within both legumes and rhizobia to sustain metabolic function at rates which do not limit growth (O'hara *et al.*, 1988; Weisany *et al.*, 2013).

2.4.1. Sulphur

Sulphur (S) is the fourth major element required for plant growth next to N, P, and K and most crops absorbs as much S as it absorb P. Sulphur deficiency has been reported in the last years even in many previously sulphur sufficient areas of the world. Scherer (2009) reported that S is becoming deficient due to cultivation of high yielding variety, use of high grade S free fertilizer, and absence of industrial activities. Eriksen *et al.* (2004) also indicated that less S is being added to soils due to the decreasing use of S-containing fungicides, pesticides, and due to the reduction of sulphur dioxide emission from industrial sources (Scherer, 2001; Eriksen *et al.*, 2004). Tandon (1989) reported that when S is deficient in soil, full yield potential of the crop cannot be realized even in good crop husbandry practices.

S plays a great role in plant metabolism. It constitutes the main element of amino acids (cysteine and methionine), which are of essential nutrient value and needed for protein synthesis (Jan *et al.*, 2002). Ferro-sulphur proteins play an important role in nitrogen fixation and electron movement in photosynthesis (Kadıoğlu, 2004). Katyal *et al.* (1987) also reported that the nutrition value of cereals is determined by the proportion of S containing amino acids. Leguminous plant species require a large quantity of S, probably because of their high protein content. Average S removal for producing 1 tone of food grain is estimated to be 3-4 kg by cereals (wheat and rice), 5-8 kg by sorghum and millet, 8 kg by pulses and legumes and 12 kg by oilseeds (Kanwar and Mudahar, 1985). Therefore, S deficiency in legume crops affects yield formation, quality and the nutritional value of seeds (Sexton *et al.*, 1998). This is mainly because methionine is usually the most limiting essential amino acid in legume seeds (Friedman, 1996).

Moreover, S has important function in reduction of CO_2, formation of chlorophyll and production of organic compounds (Scherer, 2008). Photosyntehtic product is the ultimate source of carbon for both N_2 fixation and assimilation (Vance *et al.*, 1998). Kacar (1984) reported that S has positive effects on root growth in plants and positively affects nodulation in legume crops in particular. S is also a vital part of the ferredoxin, an iron-sulphur protein occurring in the chloroplasts. Ferredoxin has a significant role in nitrogen dioxide and sulphate reduction, the assimilation of N by root nodule bacteria and frees living N-fixing soil bacteria (Scherer *et al.*, 2008).

Legume crops obtain N mainly from symbiotic N_2 fixation, which may be affected by S deprivation. Scherer and Lange (1996) found a lower N accumulation and a yield reduction when S was limiting. S application and inoculation have immense potentional of increasing the amount of N fixed by legumes, thus improving fertility status of soil (Habtegebrial *et al.*, 2007). Lange (1998) suggests that S affects growth of leguminous plant through its effect upon N_2 fixation by *Rhizobium* microorganisms. Because of relatively high S content of the nitrogenase (Mortensen and Thornley, 1979) and of ferredoxin (Yoch, 1979), S deficiency may affect N_2 fixation. Growth and nitrogen (N) fixation rates by legume could be increased by highly efficient, competitive and persistent strains of *Rhizobium* (Amanuel *et al.*, 2000). In addition, supply of adequate amount of P and S increased this process (Olivera *et al.*, 2004; Scherer *et al.*, 2008). According to Muhammad *et al.* (2013). Application of both phosphorus and sulphur resulted in increase in nitrogen fixation up to 38% and 33% over control, respectively. Nutrient uptake of nitrogen, phosphorus and S increased significantly with the application of P and S and positively correlated with nitrogen fixation. The same author also reported that, there is direct involvement of sulphur in the process of nitrogen fixation whereas effect of phosphorus on nitrogen fixation is indirect mainly through enhanced growth and dry matter production. Togay *et al.* (2008) also reported that chickpea variety applied with phosphorus, sulphur and inoculation resulted in higher grain yield. S application significantly increased the uptake of Fe, Mn, Zn and Cu in grain in the both years. Despite the importance of this element in crop production, it is still not included in fertilizer recommendations of Ethiopia especially for legume crops like chickpea.

2.4.2. Zinc

Zinc (Zn) has an important metabolic role in plant growth and development and therefore, called an essential trace element or micronutrient (El Habbsha *et al.*, 2013). Zinc is involved in various host plant metabolic processes, nodule growth and N_2 fixation processes. Zn uptaken and transfered in the form of Zn^{2+} in plants and an essential nutrient that has particular physiological functions in all living systems, such as the maintenance of structural and functional integrity of biological membranes and facilitation of protein synthesis and gene expression, enzymes structure, energy production and Krebs cycle; also has a positive impact on crop yield (El Habbsha *et al*., 2013). In addition to having an important role in activating plants enzymatic systems. Zn is essential for the synthesis of chlorophyll and carbohydrates. This element plays an important role in the metabolism of nitrogen, synthesis of amino acid tryptophan, metabolism of starch, plants flowering and fruit set, increasing plant resistance to fungal disease and expanding plant roots (Bahure *et al*., 2016).

Zn solubility decreases markedly above pH 6.0-6.5 (Sims, 2000) and thus, Zn, deficiencies can be encountered in neutral to alkaline soils (Roy *et al*., 2006). Zn deficiency in soil is one of the most important factors reducing production of such plants as corn, soybean, bean, rice and wheat. Not only Zn deficit reduces these crops yields and production, but also results in reduction of their nutritional value (Bahure *et al*., 2016). In Zn deficient plants, protein synthesis and protein levels are markedly reduced, but amino acids and amides are accumulated as Zn is the structural component of the protein synthesizing polymerase enzyme. Hence, in Zn deficient plants, the protein synthesis of Ribonucleic Acid (RNA) is impaired (Fageria, 2009).

In legume plant, deficiency of Zn is found to reduce the number and size of nodules as it is possibly involved in leghaemoglobin synthesis. Moreover, Zn deficiency resulted in delay in crop maturity, reduces water use and water use efficiency (Khan *et al*., 2004), nodulation and nitrogen fixation (Ahlawat *et al*., 2007), inturn reduced crop yield. Zn uptake is positively correlated with the amount of organic matter in the soil and negatively correlated with P concentration in the soil (Hamilton *et al*., 1993; Ahlawat *et al*., 2007).

Zn is the main micronutrient limiting chickpea productivity (Fageria, 2009). Zn deficiency is common in the chickpea growing regions of the world and is perhaps the most widespread of micronutrient deficiencies (Roy *et al*., 2006; Ahlawat *et al*., 2007). Chickpea is generally considered sensitive to Zn deficiency (Khan, 1998). Many researches indicate that application of Zn has a positive role in the nodulation and grain yield of legumes . According to Bahure *et al*. (2016), application of Zn, Fe and Mn significantly affect yield parameters of soybean and they conclude that this is due to better uptake and translocation of plant nutrients to growing plants and more photosynthesis which in turn promoted more number of leaves, leaf area and dry matter production. Valenciano *et al*. (2011) also reported that plants fertilized with Zn and with Mo had a greater total dry matter production and seed yield of chickpea, mainly due to an increment in pod dry matter. The highest yield was obtained with 2 mg Zn per plant.

Abdel-Salam (1986) as cited by Abdel-Ghaffar (2009) showed that foliar application of Zn to faba bean plants with and without nitrogen fertilization increased nodule number and dry weight, nitrogenase activity, dry weight of plants, and plant uptake of N and P. A research conducted in Tigray showed an increasing trend in nodule number and dry weight with increasing Zn fertilization (Weldu and Habtegriel, 2013). Similarly, the combined fertilization of P and Zn fertilizers showed significant effect on P, Zn and N concentration of plant leaves.

An important aspect of P and Zn nutrition is the interaction effect between them, especially in soils marginally deficient in P and Zn. If P and Zn are fertilized together in such soils, crop yields would be increased with positive interaction of P and Zn (Havlin *et al*., 2005). However, high P availability or fertilization of P alone is found to induce Zn deficiency in plants, commonly known as P induced Zn deficiency (Cakmak and Marschner, 1987). At high P availability, the physiological availability of Zn is decreased, where its solubility and mobility both within the cell and in long distance transported to the shoot apex is also affected. Furthermore, with Zn deficient plant, cellular regulation of P uptake is impaired, causing absorption of toxic levels of P and transportation to plant tops, creating symptoms resembling Zn deficiency (Havlin *et al*., 2005). High soil P availability or fertilization also increases the shoot to root ratio of plants, resulting in short root length, thus, suppressing mycorrhizal uptake of Zn, which is the major Zn acquisition process by plants.

3. MATERIALS AND METHODS

3.1. Description of the Study Areas

3.1.1. Gondar Zuria District

Gondar Zuria is located 730 kms Northwest of Addis Ababa in Amhara National Regional State. It is one of the sixteen Woredas of North Gondar Zone of the Amhara National Regional state. It is bordered in the south by the Debub Gondar Zone, in the southwest by Lake Tana, to the west by Dembiya, to the north by Lay Armachiho, to the northeast by Wegera, and to the southeast by Belessa. Towns in Gondar Zuria district include *Degoma, Emfraz, Maksegnit* and *Teda* (CSA, 2005). The total area of this district is 114983 ha of which, 38830 ha^{-1}, 11073 ha^{-1}, 16851 ha^{-1}, 17016 ha^{-1} and 2065 ha^{-1} of the Woreda were covered by agricultural land, forest land, bush land, grazing land and un-cultivated land, respectively (GOZOARD, 2016).

Agro ecologically, the altitude gradient of Gondar Zuria District is within the range of 1107-3022 m a.s.l, and have three agro ecological zones. The two agro ecology zones, *Weynadega* (1500 - 2300 m a.s.l) and *Dega* (2300-3200 m a.s.l.) constitute the largest area coverage as compared to the *Kolla* (GOZOARD, 2016). According to GOZOARD (2016) in the district (*Maksegnit*) mean annual temperature ranges between 14-20°C with the mean of 17.9°C (From 11 year collected data from *Maksegnit*). The rainfall varies between 1030-1223 mm with the mean annual rainfall of 1100 mm (According to 18 year collected data from *Maksegnit*). The soils of the district are Litic Luvisols (49%), Humic Nitisols (10%), Haplic Luvisols (12%), Eutric Vertisols (16%) and Chromic Luvisols (13%) (GOZOARD, 2016)

Specifically, this on farm exprirment was conducted at Tsion Kebele ($37^0$33'33.9"E-$37^0$33'34.1"E longitude and $12^0$25'00.9"N-$12^0$25'00.93"N latitude with an elevation of 1924m) and Das Denzaz Kebele ($37^0$36'24.9"E-$37^0$36'25.01"E longitude and $12^0$25'08.1"N-$12^0$25'08.13"N latitude with an elevation of 2037m).

3.1.1.1. Tsion Siguaje Kebele

Tsion kebele is located at 1 km away from Woreda Town Maksegnit. Agro ecologically, it is categorized under *Woynadega*, with altitude range between 1800-2000 m.a.s.l. The total land area of the district is 1963.37 ha^{-1} and of which, agricultural land shares 1143 ha^{-1}. The dominant crops being cultivated in this district are sorghum, Tef, Chickpea, Maize, Wheat and, Barley (GOZOARD, 2016). According to GOZOARD (2016) the dominant soil type covering 80 % is Vertisols followed by 15% Nitisols and 5% Cambisols.

3.1.1.2.Das Denzaz Kebele

This Kebele is located at 12 km away from woreda town Maksegint. Agro ecologically, it is categorized as *Woyenadega*. According to GOZOARD (2016) the dominant soil type covering 64 % is Cambisols followed by 21% Nitisols and 14.5% Vertisols. From the total area of the Kebele, the share of agricultural land is 1486 ha^{-1} (43.7 %). Tef, wheat, sorghum, chickpea, barley, and potato are the major crops cultivated in this kebele. Intercropping barley with sorghum and barley with lentil are practiced in the area.

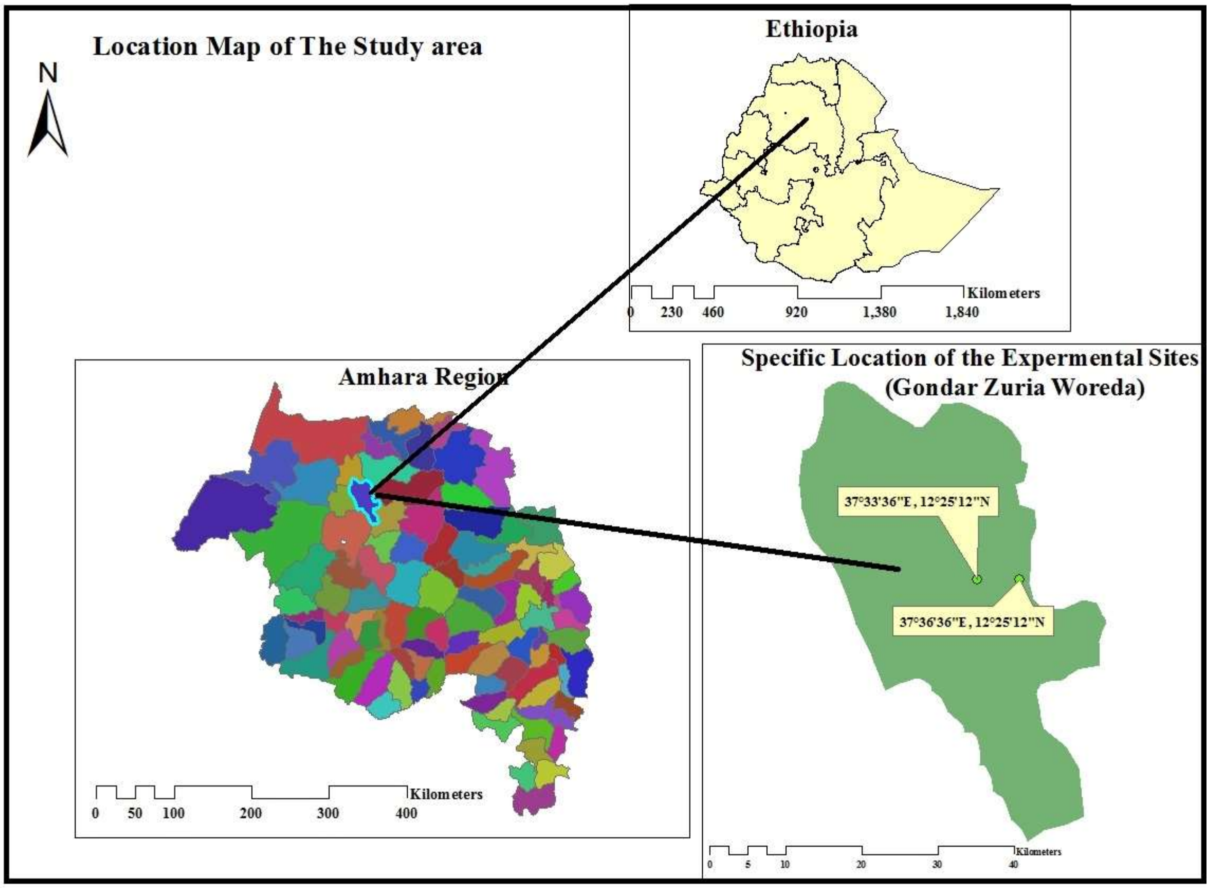

Figure 1. Location map of the study area

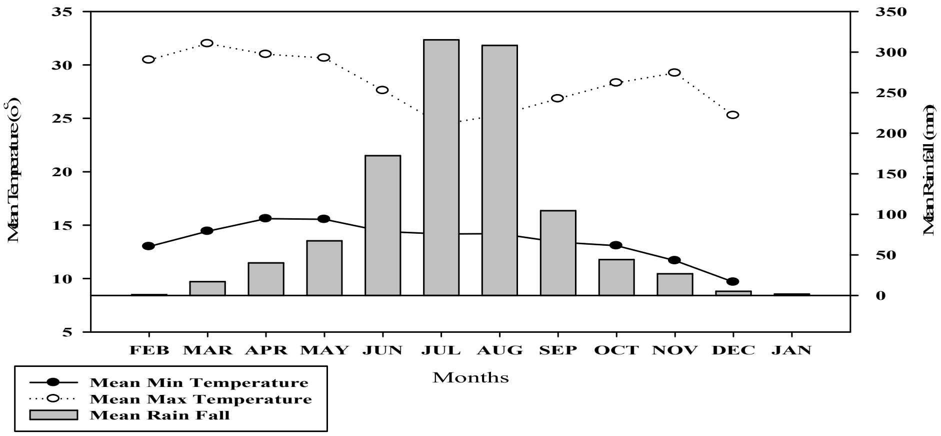

Figure 2. Long term meteorological data of Maksegnit

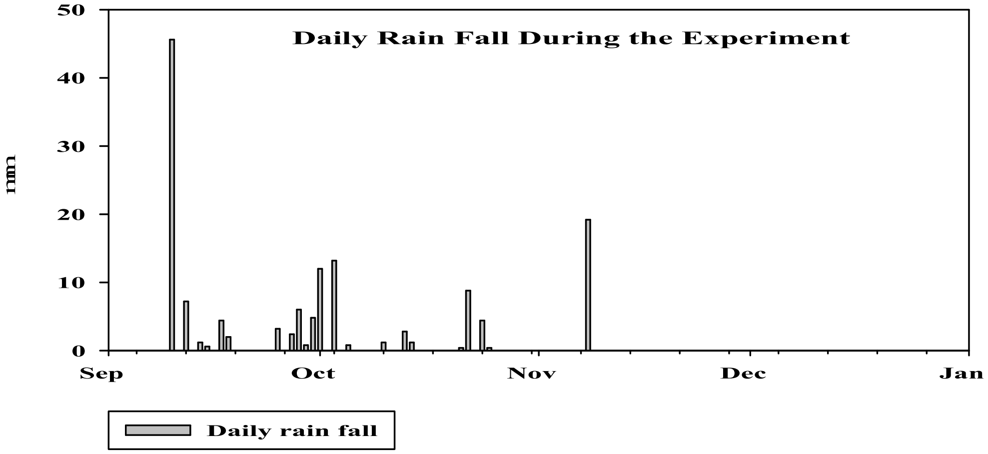

Figure 3. Daily rain fall distribution during the experiment (planting to late pod setting stage)

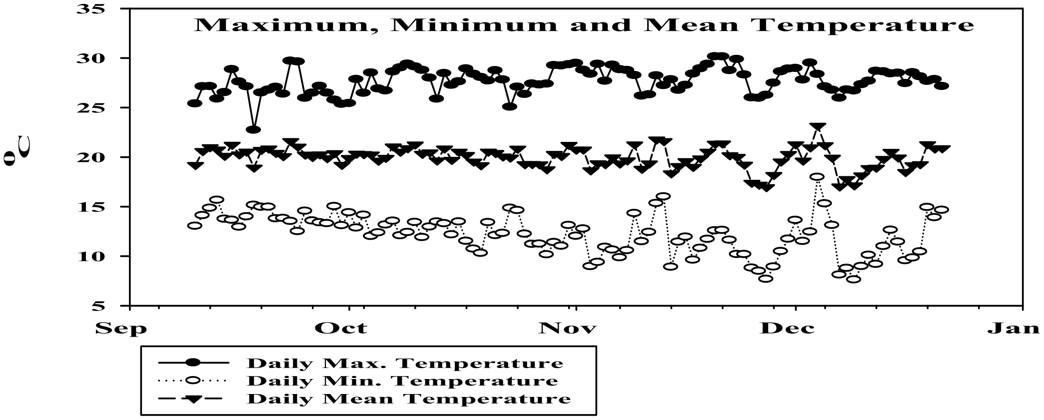

Figure 4. Daily max, min and mean temperature during the experiment (planting to late pod setting stage)

3.2. Experimental Details and Data Collection

3.2.1. Soil and Plant Sample Collection and Processing

To identify the possible yield limiting essential nutrient in the study sites, soil samples were collected from those experimental sites on which diagnosis and demonstration of P fertilizer and *Rhizobium* inoculation trial were implemented in 2014/15 cropping season. Five separate composite surface soil samples (0-30 cm depth) were collected from Gondar Zuria woreda (Tsion, Denzaz and Degola Chenchya kebele) from 10 sampling spot of the entire experimental site before planting for determination of the physico-chemical properties of the soil and hence for identification of the limited plant nutrients. Soils were air dried, ground and mixed thoroughly and passed through a 2 mm sieve for most parameters except for OC and TN which passed through 0.5 mm sieve. The samples were then labeled and stored in sealed plastic bags for laboratory analysis of; texure, pH, TN, OC/OM, CEC, exchangeable cations (Ca, Mg, K, and Na) extractable P, extractable S, and micronutrients (Zn, Fe, Cu and Mn).

At physiological maturity, five randomly selected plants were harvested at the ground level and partitioned in to grain and straw. The plant material was dried to a constant weight in a forced-draft oven at 70°C to a constant weight, grounded and passed through 1 mm sieve for determination of N and P concentration in grain and straw.

3.2.2. Determination of Soil Physico-Chemical Properties

Soil particle size distribution was determined by hydrometer method (Bouyoucos, 1951). Soil pH was measured with digital pH meter potentiometerically in supernatant suspension of 1:2.5 soil to distilled water ratio (Van Reeuwijk, 1992). Cation exchange capacity (CEC) was determined by 1M ammonium acetate method at pH 7 (Chapman, 1965) whereas organic carbon (OC) was determined by the dichromate oxidation method (Walkley and Black, 1934). Total N in the soil was measured by the micro kjeldhal method (Jackson, 1958). Available P was analyzed by Olsen method (Olsen *et al*., 1954) colorimetrically by the ascorbic acid- molybdate blue method (Watanabe and Olsen, 1965).

Available S was analyzed by FAO-turbidimetric method (Ajwa and Tabatabai, 1993). From 1 M ammonium acetate leacheate, Exchangeable Ca^{++} and Mg^{++}were measured by Atomic

Absorption Spectrophotometer while exchangeable Na^+ and K^+ were determined by flame photometer. Micronutrient (Zn, Mn, Fe and Cu) were measured using Dietylene Triamine Penta Acetic Acid (DTPA) extraction following the procedure developed by Lindsay and Norvell as outlined by Sahlemedhin and Taye (2000). Finally, the status of those nutrients which are essential for BNF were rated. The treatments were developed based on those nutrients rated as low in the study sites.

3.2.3. Plant Sample Analysis

For plant sample analysis, grounded material (only tops) was digested with a 2:1 mixture of nitric (HNO_3) and perchloric acids ($HC1O_4$) for P determination. Phosphorus concentration in the shoot was analyzed colorimetrically (Morais and Rabelo, 1986). Nitrogen content was determined using Modified micro-Kjeldahl Method (Jackson, 1958). About 0.25 g for grain samples, and 0.50 g for straw were taken for analysis. The total N and P in the grain and straw were finally expressed in percentage.

3.2.4. Enumeration of Indeginous Rhizobia Nodulating Chickpea

The numbers of indigenous rhizobia nodulating chickpea *(Cicer artenium* L.*)* present in the soils of the study sites could nodulating chickpea was estimated by the most-probable-number (MPN), plant infection technique following Somasegaran and Hoben (1994). For this purpose, soils were collected two days ahead of planting from the top 20 cm from five locations and bulked to one composite sample per farm. The samples were brought to laboratory and stored in a refrigerator at 4°C until pot experiment started.

This experiment was conducted under semi-controlled greenhouse at Haramaya University. Uniform size, high viability and healthy seed of chickpea var. Arerti was used. Seeds were surface sterilized with 95% of ethanol and in 3% (v/v) solution of sodium hypochlorite. The seeds were successively rinsed in sterilized distilled water several times. The sand was sterilized in dry oven at 160 ^{O}C for 1.5 hrs two times. The sterilized sand was added to plastic pot. Three sterilized seeds were planted to each plastic pot and allowed to germinate. After germination, two seedlings were removed and one health seedling was maintained for nodulation scoring.

A tenfold serial dilution was prepared by adding 1 gram of soil in to 9 ml of distilled water and sequentially diluting 1 in 10 to give a dilution series to 10^{-10}. The pots were inoculated with each of the ten serial dilutions from the soil by using 1 ml aliqutos. Each dilution was replicated four times. The plants were frequently inspected and water was provided periodically. After three weeks, they were carefully uprooted and the numbers of nodulated pots were recorded. The numbers of rhizobia were calculated using the following formula:

$$X = \frac{m \, X \, d}{v}$$

Where:

m = Likely number from the MPN table for the lower dilution of the series

d = Lowest dilution (first unit used in the tabulation)

v = Volume of aliquot applied to plant

X = The MPN per gram of inoculant

3.2.5. Field Trial on The Study Site

3.2.5.1. Expermental Design and Treatments

The experiment comprised of three factors with two levels of *Rhizobium* inoculation (R_1 = *Rhizobium* inoculated and R_0 = Un-inoculated), three levels of sulphur (0, 15 and 30 kg S ha^{-1}) (Muhammad *et al*., 2013) and two levels of Zn (0 and 1.5 kg Zn ha^{-1}) (Valenciano *et al*., 2009). The factorial combinations of the three factors (2*3*2 = 12) were laid in randomized complete block design with three replications. In addition, the negative control (without fertilizer and *Rhizobium* inoculation) was included to determine the P use efficiency.

Treatment combinations used in the experiment

1. *Rhizobium* inoculation alone
2. *Rhizobium* inoculation +15 kg ha^{-1} Sulphur
3. *Rhizobium* inoculation + 30 kg ha^{-1} Sulphur
4. Control check
5. 15 kg ha^{-1}Sulphur alone
6. 30 kg ha^{-1}Sulphur alone
7. *Rhizobium* inoculation + 1.5 kg ha^{-1} Zinc
8. *Rhizobium* inoculation + 15 kg ha^{-1}Sulphur + 1.5 kg ha^{-1} Zinc
9. *Rhizobium* inoculation + 30 kg ha^{-1} Sulphur + 1.5 kg ha^{-1} Zinc
10. 1.5 kg ha^{-1} Zinc alone
11. 15 kg ha^{-1}Sulphur +1.5 kg ha^{-1} Zinc
12. 30 kg ha^{-1}Sulphur + 1.5 kg ha^{-1} Zinc
13. Negative Control (without fertilizer (including starter N and P) and *Rhizobium* inoculation)

3.2.5.2. Land preparation

Land preparation (ploughing, and leveling) was done based on the recommendation given to the crop.

3.2.5.3. Source of Rhizobial Isolates

Rhizobium ciceri strain CPM41 that was selected based on its ability to enhance nodulation and grain yield under wide ecological condition was obtained from MBI (Menagesha Biotechnology Industry).

3.2.5.4. Source of Improved Seeds

The chickpea variety “Arerti” was used as test variety. The variety was selected based on the recommendation of Gondar Agricultural Research Center (GARC) for the area and the seeds were received from extension and economics department of GARC.

3.2.5.5. Method of Seed Inoculation

Seed inoculation was performed before sowing using the procedure developed by Fatima *et al.* (2007). To ensure the sticking of the applied inoculant to the seeds, the required quantity of seed was suspended in 1:1 ratio in 10% sugar solution. The inoculant was gently mixed with dry seeds at the rate of 10 g per kg of seed. Inoculation was done just before sowing under shade to maintain the viability of cells and allow to air dry for a few minutes and then the inoculated seeds were sown at recommended rate and spacing to the respective plots. To avoid contamination, plots with un-inoculated seeds were planted first followed by the innoculated ones.

3.2.5.6. Sowing

The plot size used was 3 m x 3.4 m (10.2 m^2). Seeds were sown in rows by maintaining 30 cm and 10 cm between the rows and plants, respectively. There were 10 rows per plant and 34 plants in each row. A net plot size was 3.4 m x 1.8 m (6.12m^2) was for the final harvest. The spacing betweeb each plot and block were 1 m and 1.5 m, respectively.

The planting was done on September 13 and 15/2016 at Denzaz and Tsion, respectively. To maintain the population in each treatment, two seeds per hill were planted and thinned to a single plant per hill after two weeks of germination. Ridges were made between each plot and block to reduce the movement of bacteria and fertilizer from one plot to the other by rain. Weeding and fungicide spray were done regularly to keep the experimental plants free of weed and disease.

3.2.5.7. Fertilizer Application

All treatments (except the negative control) received equal amount of starter inorganic 20 kg N ha^{-1} (Anteneh and Daniel, 2016), 20 kg P ha^{-1} (Ahlawat and Ali, 1993; Ramakers, 2001) in the form of Urea and Triple super phosphate, respectively. Different rates of calcium sulfate/gypsum and zinc sulfate were applied as indicated in the treatments. Zinc sulfate was applied on foliar parts (El-Habbasha *et al*., 2013; Pathak *et al*., 2012). The remaining fertilizers were applied directly to the soil at the time of planting (Corp *et al.,* 2004).

3.2.6. Data Collection

3.2.6.1. Data Collected at Late Flowering Stage

Sampling for nodulation was performed by excavating the roots of plants randomly from two rows next to boarder rows of each plot at the mid flowering stage of the crop. Uprooting was done by spade and shovel and soil was removed from the root system by hand. The adhering soil was removed by washing the roots gently with water over a metal sieve. Nodules remaining in the soil were picked up by hand. The plants from each plot were used to record the following observations.

Nodule rating: Nodulation rating was done by careful uprooting of five plants with intact nodule. The plants were examined for nodulation in the tap root, in the secondary root but close to the tap root, scattered all over the root and plants showing no root nodulation. The rating of the plant for nodulation was done in scale of 1-10. The nodule rating was done following the formula mentioned in NifTAL, (1985).

$$\textbf{Nodulation rating} = \frac{(\mathbf{10} \text{ x } \boldsymbol{NPTRN})+(\mathbf{5} \text{ x } \boldsymbol{NPNCTR})+(\mathbf{1}\text{x } \boldsymbol{PSN})+(\mathbf{0} \text{ x } \boldsymbol{PNN})}{\boldsymbol{N}} \text{...........}\textbf{(1)}$$

Where,

NPTRN: Number of plants with tap root nodulation

NPNCTR: Number of plants with nodules in secondary root but close to taproot

PSN: Number of plants with scattered nodulation

PNN: Number of plants without nodulation

N: Total number of plants

Number of Nodules: These were determined by counting the number of nodules from five plants and the mean value of the five plants were recorded as number of nodules per plant.

Nodule volume: The collected nodules were immersed in previously measured volume of water in measuring cylinder. The volume of water displaced by nodules was considered as nodule volume (ml).

Number of effective nodules: Ten representative nodules were taken from five up rooted plants from each plot and dissected with blade to observe their color in the center. The color score were made in 1-4 scale as: 1 = white, 2 = pink, 3 = slightly dark red and 4 = deep dark red as adopted by Tekalign and Asgelel (1994).

Nodule dry weight: The collected nodules were labeled and placed in perforated paper bags. The nodule dry weight per plant was measured after drying the collected nodules in an oven with a temperature of 65ºC for 24-48 hrs until constant weight is attained. The average of five plants was taken as a nodule dry weight per plant.

3.2.6.2. Data Collected at Early Pod Seeting Stage

Shoot length: At late flowering stage, three plants were up rooted from each middle plots and the shoot length was measured. The mean from three plants was used as shoot length.

Shoot dry weight: After measuring the shoot length, the plants were kept at 65ºC in oven until getting constant weight. The mean value from three plants was taken as shoot dry weight per plant.

3.2.6.3. Phenological Data

Days to 50% flowering: It was determined as the number of days after seedling emergence to the period when 50% of the plants in a plot developed first flower.

Days to maturity: It was taken as the number of days after seedling emergence to the period when 90% of the plants in the plot were ready for harvesting as revealed by change in the foliage and pod color and seed hardening in the pod.

3.2.6.4. Yield and Yield Component Data Collected at Harvest

Number of pods per plant: It was recorded from ten randomly selected plants from the net plot area at harvest. The average result was reported as number of pods per plant.

Number of seeds per pod: It was determined from randomly selected five pods from the plants used for pod number count from the non-boarder plots. The average number of grain per pod was calculated by dividing the total number of grains with the number of pods per plant

3.2.6.5. Data Collected After Harvesting

Above ground biomass yield (kg ha^{-1}): At physiological maturity, plants from central row were manually harvested close to the ground surface. The harvested plants were sun-dried in an open air, weighed to determine the above ground biomass yield**.**

Grain yield (Kg ha^{-1}): It was determined after threshing and adjusting the grain yield at the appropriate moisture level of 10.5%. Finally, yield per plot was converted to per hectare basis.

Hundred seeds weight (g): It was determined by weighing 25 randomly selected grains and weighing with sensitive balance and multiplying by four. It was reported as 100 grain-weight.

Straw yield (Kg ha^{-1}): It was calculated by subtracting grain yield from the corresponding total above ground biomass yield.

Harvest index (HI): It was computed as the ratio of seed yield to biomass yield.

3.2.6.6. Estemation of Total N and P uptake

Phosphorus Uptake by seed and straw was determined from the P content of respective part after multiplying the seed yield and straw yield, respectively. Similarly the N uptake by seed and straw was determined from the N content of respective part after multiplying the seed and straw yield, respectively. Total N and P uptake were calculated by adding the N and P uptake of seed and straw.

3.2.6.7. Estimation of Phosphorus Harvest Index and Phosphorus Use Efficiency

3.2.6.7.1. Estimation of phosphorus harvest index and phosphorus use efficiency due to phosphorus application

Phosphorus harvest index (PHI) and phosphorus Use Efficiency (PUE) were calculated with the help of the following formula

$$\mathrm{PHI} = \frac{\text{P uptake in grain}}{\text{P uptake in grain + straw}} \quad \text{(Fageria and Santos, 2002)}$$

$$\mathrm{PUE} = \frac{\text{grain and straw yield at higher P level} - \text{grain and straw yield at lower P level}}{\text{P uptake in grain and straw at higher P level} - \text{P uptake in grain and straw at lower P level}}$$

(Fageria and Santos, 2002)

Where

PHI = Phosphorus harvest Index

PUE = Phosphorus Use efficiency

3.2.6.7.2. Estimation of phosphorus use efficiency due to application of sulphur and zinc

Phosphorus Use efficiency due to S and Zn fertilization was calculated with the help of the following formula

$$\text{PUE} = \frac{\text{grain and straw yield at higher S and Zn level} - \text{grain and straw yield at Zero S and Zn level}}{\text{P uptake in grain and straw at higher S and Zn level} - \text{P uptake in grain and straw at Zero S and Zn level}}$$

Where

PUE = Phosphorus Use efficiency

3.2.6.8. Estimation of total P and N uptake

3.2.7. Data Analysis

The collected data were subjected to three factors analyses of variance (ANOVA) to evaluate the main and interaction effect of the factors (fertilizers and inoculation) on the selected parameters using SAS 9.1 statistical software. Where ever the treatment effect were significant, mean separation were made using the least significance (LSD) test at 5% level of probability. Correlation between parameters were computed when applicable according to Gomez and Gomez (1984).

3.2.8. Economic Analysis

Based on procedure described by CIMMYT (1988), economic analysis was done using partial budget analysis. For partial budget analysis, the variable cost of fertilizer and labor were taken at the time of planting and during other operations. Price of the grain and straw yield of chickpea were considered. The cost of *Rhizobial* inoculant was also considered. The average yield was adjusted down ward by 10 % to reflect the farmer's field yield as described by CIMMYT (1988). The return was calculated as total gross return minus total variable cost. Field seed price (22.5 Birr kg^{-1} seed), field price of inoculant (240 Birr ha^{-1}), field straw price (2.00 Birr kg^{-1}) of the average of one month from the time of crop harvesting and farm-get prices of zinc sulfate fertilizer (15.00 Birr kg^{-1}) and market price of calcium sulfate (2.4 Birr kg^{-1}) during planting time and labor cost at (40 Birr per person per day) were used for variable cost determination. All input for economic analysis was based on mean value over location.

Net benefits and costs that vary between treatments were used to calculate marginal rate of return to invested capital as we move from a less expensive to a more expensive treatment. Before conducting marginal analysis of all treatment, net benefit curve was established by putting variable cost at X axis and net benefit at Y axis. Regression line was added on the curve. Any points below the regression line were identified as dominated and hence dropped. Then marginal analysis of un-dominated treatment were performed to identify the one that will be economically attractive to farmers (CIMMYT, 1998). To draw farmers' recommendations from marginal analysis in this study, 100% return to the investments was used as reasonable minimum acceptable rate of return.

4. RESULTS AND DISCUSSION

4.1. Physico-chemical Properties of the Soils of the Study Sites

4.1.1. Soil Physical Properties

4.1.1.1. Texture

Soil texture is one of the inherent soil properties less affected by management and which determines nutrient status, organic matter content, air circulation and water holding capacity of a given soil. Based on the soil analysis made, the soil texture of the entire sites was clay. This soil is characterized by high water holding capacity. Due to this, farmers of the study area plant chickpea on residual soil moisture starting from the first to last week of September.

4.1.2. Soil chemical Properties

4.1.2.1. pH of Soil

The results of the selected soil physical and chemical properties are presented in Table1. According to the rating by Tekalign (1991), the pH of the experimental soils ranged from neutral (pH 7.0) to moderately alkaline (pH 7.9) (Table 1). The correlation analysis also revealed that there was a positive and significant (R^2= 0.89) relationship between soil pH and Ex.Ca. The correlations with the other soil properties were non-significant (Appendix Table 8). The result is in agreement with the findings of Fassil and Charles (2009) who reported positive and significant correlation between pH and total N, EC and Ex.Na and negative correlation with Cu.

4.1.2.2. Soil Organic Matter

Soil OM arises from the debris of green plants, animal residues and excreta that are deposited on the surface and mixed to a variable extent with the mineral component (White, 1997). According to Tekalign (1991), the entire site had low OM content (Table 1). This is because of continuous cultivation without returning residue to the soil. Similarly, Fassil and Charles, (2009) reported that vertisols of Ethiopia had low soil OM content. Other authors also reported low soil OM in Vertisols (Kamara and Haque, 1987; Giday *et al*., 2015; Kiflu and Beyene*,* 2013).

4.1.2.3. Total Nitrogen

Nitrogen (N) is the fourth plant nutrient taken up by plants in greatest quantity next to C, O and H, but it is one of the most deficient elements in the tropics for crop production (Mesfin, 1998). It has been observed in Table 1, that total N in the study sites varied from 0.04% to 0.07% with a mean value of 0.052%. Based on Tekalign (1991), total nitrogen content of site two was very low while the remaining sites was low (Table 1). This result is in line with the previous findings of many scholars who reported that N is one of the most deficient elements in the tropics for crop production (Finck and Venkateswarlu, 1982; Mengel and Kirkby, 1987; Mesfin, 1998; Hillette *et al*., 2015).

4.1.2.4. Extractable Phosphorus

Phosphorus (P) is known as the master key to agriculture next to N because lack of available P in the soils limits the growth of both cultivated and uncultivated plants (Foth and Ellis, 1997). Olsen extractable P content of the soil in the experimental sites ranged from 0.8 to 17.1 mg kg^{-1} with a mean value of 7.42 mg kg^{-1} (Table 1). According to Landon (1991), the available P was rated as low for sites 1 and 3, medium for sites 4 and 5 and high for site 2. The source of variation across farms may be due to the different history of fertilizer usage (especially DAP) and the inherent soil variability across farms.

4.1.2.5. Available Sulfur

Sulfur is an important secondary nutrient which is responsible for synthesis of cysteine, methionine, chlorophyll, vitamins, metabolism of carbohydrates, oil and protein contents (Sarkar *et al*., 2002; Singh *et al*., 2006). According to Lewis (1999) S content of all study sites ranged from very low to low (Table1). The low S was also expected because the experimental soil had low organic matter content (source of about 95% of S) indicating that its potential to supply S to plant growth through mineralization is low. EthioSIS soil fertility map showed that Sulfur content of almost all soils of Gonder Zuria woreda is in very low to low range (EthoSIS, 2016). Similarly, Lelago *et al.* (2016) reported that among soil samples collected from Kacha Bira woreda, 88.43%, 10.2% and 1.37% were very low, low and optimum in S content respectively. Moreover, 85.1% of soils of Damboya were very low in S content while the remaining 14.19% was low in S content. Other authors also reported deficiency in S in Vertisols (Hillette *et al*., 2015; Fanuel, 2015; Habtamu *et al*., 2014)

4.1.2.6. Cation Exchange Capacity

The cation exchange capacity (CEC) of soils is defined as the capacity of soils to adsorb and exchange cations (Brady and Weil, 2002). According to the rating developed by Hazelton and Murphy (2007), the soils of the investigated sites had very high CEC (Table 1). The result is within the range reported by Berhanu (1985), who found CEC of 35-70 meq 100 g^{-1} soil for nearly all the Vertisols of Ethiopia. The very high value of CEC is mainly due to the high clay content of all sites. CEC of soil is an important parameter of soil because it gives an indication of the type of clay minerals present in the soil, its capacity to retain nutrients against leaching and assessing their fertility and environmental behavior. Generally, the chemical activity of the soil depends on its CEC.

4.1.2.7. Exchangeable Bases

According to Fassil and Charles (2009), in neutral Vertisols, the exchangeable sites are occupied mainly by calcium (Ca) and magnesium (Mg) and to a lesser extent by potassium (K) and sodium (Na). The present study (Table1) also confirmed that 64.9%, 60.8%, 75.9%, 67.2%, 61.9% of the exchangeable sites of the study sites 1, 2, 3, 4 and 5 were occupied by Ca respectively, indicating that Ca is the dominate cation in the cation exchange sites. Similar result is reported by Hillette *et al*. (20015) for Vertisols cropping system of central highlands of Ethiopia. Next to Ca, the exchangeable sites were occupied by Mg, K and Na in all sites. Similarly, Hillette *et al.* (2015) found that 77% of the exchangeable site were occupied by Ca^{++} followed by Mg^{++} (19%) and K^{+} (3.2%). Exchangeable K in the study area ranged from 0.6 cmol (+) K kg^{-1} in site 1 to 1.1 cmol (+) K kg^{-1} in site 2. According to Berhanu (1985) all sites had high exchangeable K. This result is also in agreement with different former findings (Beyene, 1982; Kamara *et al*., 1989; Lemma and Smit, 2008; Hillette *et al*., 2015).

Exchangeable Ca varied between 39.2 cmol (+) Ca kg^{-1} in site 5 to 56.7 cmol (+) Ca kg^{-1} in site 3. According to Hazelton and Murphy (2007) all sites had very high exchangeable Ca. Similarly, research in different part of Ethiopia indicated that Vertisols have high Ca content in their exchange sites (Beyene, 1982; Kamara *et al*., 1989; Lemma and Smit, 2008; Fassil and Charles, 2009; Hillette *et al*., 2015).

Exchangeable Mg in the study sites varied from 16.9 cmol (+) Mg kg^{-1} in site 3 to 27.1 cmol (+) Mg kg^{-1} in site 1. Based on the rating developed by Hazelton and Murphy (2007) all sites were very high in their exchangeable Mg content. Similarly, Hillette *et al*. (2015) reported that all samples collected from 10 locations having clayey texture and neutral to slightly alkaline pH (7.2-7.9) had high exchangeable Mg^{+} which is above the critical level (1-3 cmol kg^{-1}) according to Hazelton and Murphy (2007).

4.1.2.8. Zinc

Zinc plays important role in plant metabolism and influences hydrogenase and carbonic anhydrase activities, stabilize ribosomal fractions and help in the synthesis of cytochrome (Tisdale *et al*., 1985). Zn deficiency is widespread in many of the world's major chickpea-growing areas (Cakmak *et al*., 1995). The authors reported that half of the samples collected from 25 countries found to be very low in Zn content. According to Ahlawat (2007) chickpea is more sensitive to Zn deficiency. Accordingly, the DTPA extractable Zn content in the soil ranged from 0.4 mg kg^{-1} (site 5) to 0.6 mg kg^{-1} (site 2) (Table 1). All the soil analysis results were lower than the mean value (0.9 mg kg^{-1}) reported for Vertisols by Asgelil *et al*. (2007). But similar to the mean value of available Zn (0.5 mg kg^{-1}) reported by Yifru and Mesfin (2013) for Vertisols of the central highlands of Ethiopia. According to Lindsay and Norvell (1978), all sites had below the critical value of 1.0 mg kg^{-1}. This could be due to the fact that Zn has a tendency of being adsorbed on clay sized particles (Alloway, 2008). Previous research also indicated that in neutral to alkaline soils where chickpea is usually grown, Zn deficiency can often be encountered (Roy *et al*., 2006). The result is also in agreement with Yifru and Mesfin (2013). Asgelil *et al*. (2007) also reported that 78.4% of the soil samples collected from Vertisols of Ethiopia were deficient in Zn. Other research findings also confirmed deficiency of Zn in Vertisols of Ethiopia (Bereket *et al., 2011*; EthoSIS, 2016).

4.1.2.9. Iron

The DTPA extractable Fe content in the soil varied from 8.3 mg kg^{-1} to 20.5 mg kg^{-1} (Table 1). Based on Lindsay and Norvell (1978), all study sites had adequate amount of available Fe above the critical value. In conformity with the present study Hillette *et al*. (2015) found that soil samples collected from Vertisols cropping systems of central high lands of Ethiopia have sufficient level of available Fe considering 5 mg kg^{-1} AB-DTPA extractable Fe as critical.

4.1.2.10. Manganese

The DTPA extractable Mn content varied from 11.3 mg kg^{-1} to 24.5 mg kg^{-1} (Table 1 and Figure18). All sites were above the critical value of DTPA extractable Mn (1 mg kg^{-1}) developed by Lindsay and Norvell (1978). Similarly, adequacy of Mn was reported by Hillette *et al*. (2015), Itanna (1992) and Ethosis, (2016) on vertisols of Ethiopia.

4.1.2.11. Copper

The DTPA extractable Cu content varied from 1.5 mg kg^{-1} to 2.9 mg kg^{-1} (Table 1). All sites are above the critical value (0.2 mg kg^{-1}) developed for DTPA extractable Cu (Lindsay and Norvell, 1978). Yifru and Mesfin (2013) also found that all soil samples collected from Vertisols of the central highlands of Ethiopia (Minjar-Shenkora, Ada, Gimbichu, Akaki and Lume) had sufficient copper content. Similarly, sufficiency of Cu have been reported by Yifru and Mesfin (2013), Hillette *et al*. (2015) and EthioSIS (2016). In contrary to the above findings, Asgelil *et al*. (2007) reported that 51.6% of the soil samples collected from Vertisols of Ethiopia were deficient in Cu using 2 mg kg^{-1} as critical level.

Table 1. Physico-chemical properties of the soil before planting

Parameters	Before planting					
	Sites					Mean
	1/Degola$^+$	**2/Degola$^+$**	**3/Tsion***	**4/Denzaz$^+$**	**5/Denzaz***	
pH (1:2.5 H_2O)	7.3	7.0	7.9	7.6	7.0	7.4
Organic Carbon (%)	0.61	0.61	0.73	0.68	0.74	0.67
Organic matter (%)	1.05	1.05	1.26	1.17	1.27	1.16
Total N (%)	0.05	0.04	0.07	0.05	0.05	0.052
Available P (mg kg^{-1}), Olsen	0.8	17.1	2.8	9.7	6.7	7.42
Available S (mg kg^{-1}),	7.6	8.0	10.5	5.4	7.6	7.8
Ec (dS/m)	0.09	0.07	0.13	0.09	0.09	0.1
CEC(cmo (+) kg^{-1} soil)	62.6	57.6	60.1	60.1	53.1	58.7
Na^+ (cmo(+) kg^{-1} soil)	0.2	0.2	0.3	0.2	0.2	0.2
K^+ (cmo(+) kg^{-1} soil)	0.6	1.1	0.8	0.9	0.8	0.8
Ca^{2+} (cmo(+) kg^{-1} soil)	51.7	41.4	56.7	48.6	39.2	47.5
Mg^{2+} (cmo(+) kg^{-1} soil)	27.1	25.4	16.9	22.6	23.1	23.0
Fe (mg kg^{-1} soil)	8.4	20.5	8.3	10.8	16.7	12.9
Cu (mg kg^{-1} soil)	2.7	2.5	1.5	2.9	2.5	2.4
Mn (mg kg^{-1} soil)	24.5	18.1	11.3	15.5	24.2	18.7
Zn (mg kg^{-1} soil)	0.5	0.6	0.5	0.5	0.4	0.5
Sand (%)	10	10	12	12	8	
Silt (%)	20	18	16	22	18	
Clay (%)	70	72	72	66	74	
Textural class	Clay	Clay	Clay	Clay	Clay	

$^+$=indicates farmers field on which soil samples was collected for identification of limited plant nutrients, * =indicates farmers field on which the experiment conducted

4.2. Nodulation Related Data

4.2.1. Native Rhizobia Population

The MPN test revealed that population of the indigenous rhizobia in Tsion and Denzaz was found ranged between 17 x 10^{-1} to low (<10 x 10^{-1} rhizobia cells g^{-1} soil) though the districts (Woredas) have many years of experience in chickpea production (IFPRI, 2015). This indicates the population is not abundant enough to initiate optimum nodulation and provide sufficient amount of N through BNF (Slattery *et al*., 2004) especially in Tsion site. This is because of the low organic matter content of a soil since organic matter is reservoir of metabolizable energy for microbial and faunal activity and affects stabilization of enzymatic activity (Haynes, 2008). Slattery *et al*. (2004) reported that only 7% out of 50 samples collected had sufficient effective resident populations of *Mesorhizobium ciceri* nodulating chickpea. Rupela *et al*. (1987) also indicated that chickpea *Rhizobial* populations collected from soil samples from research stations and farmers' fields in different geographic regions of India had ranging from <10 to > 10^4 rhizobia g^{-1} soil and indicated that their population vary with season, depth and cropping pattern.

4.2.2. Total Numbers of Nodules Per Plant

The main effect of inoculation was found statistically significant ($P \leq 0.01$) on nodule number at Tsion and Denzaz site. Similarly, the main effect of S and Zn was found significant at Denzaz and mean value combined over locations (Appendix Table 1). The analysis result also revealed that the two way interaction of S application with inoculation and Zn were found statistically significant both locations and mean value combined. Moreover, the two way interaction of inoculation and Zn was also significant ($P \leq 0.05$) at Tsion site (Appendix Table 1).

The three way interaction of *Rhizobium* inoculation, S and Zn was found to be significant at both locations and their means (Table 2). At Tsion site, the highest nodule number (15.7) was obtained from the combined application of 15 kg S and 1.5 kg Zn ha^{-1} while the lowest (10.9) was from *Rhizobium* inoculation and 1.5 kg Zn ha^{-1}. At Denzaz site, the highest nodule number (15.8) was obtained from the combined application of *Rhizobium* inoculation, 15 kg S and 1.5 kg Zn ha^{-1} whereas the lowest (9.3) was from the control check as well as 15 kg S alone. The highest (15.3) mean nodule number over locations was obtained from *Rhizobium* inoculation integrated with 15 kg S and 1.5 kg Zn ha^{-1} which resulted in 37.8% increment over the control check. Even if there was no consistent increase, the increase of number of root nodules with increasing levels of Zn might be due to the fact that Zn helps to improve more nodulation and leghaemoglobin formation (Brady and Well, 2009). Proper nutrition of plants with S increases the amount of glucose flowering to the roots and ATP biosynthesis (Pacyna *et al.,* 2006). In conformity with the present finding, Srivastava *et al.* (2006) reported that the combined application of *Rhizobium* inoculation with 30 kg S and 5 kg Zn ha^{-1} significantly increased number of nodules $plant^{-1}$ by 18%, 14.15% and 13% respectively compared with the control. This is mainly due to creation of favorable soil ecological condition for the growth and development of nitrogen fixing bacteria in gummer green gram. In addition, similar result were also concluded by Naidu and Ram (1995), Naidu *et al.* (1998), Awlad *et al.* (2003), Zhao *et al.* (2008), Abdalla *et al.* (2011), Muhammad *et al.* (2013), Surendra and Katiyar (2013), Kumar *et* *al*. (2014), Jadeja *et al*. (2016), Zafar *et al*. (2014), Sharifi (2016), Sipai *et al*. (2016), and Das *et al*. (2016).

Table 2. Number of nodules per plant of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Number of nodules per plant (no)**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	13.4^{cde}	10.9^{g}	10.5^{cd}	10.5^{bcd}	11.9^{cde}	10.7^{e}
	S15	11.3^{fg}	14.7^{abc}	11.1^{bc}	15.8^{a}	11.2^{cde}	15.3^{a}
	S30	14^{bcd}	11.9^{efg}	10.7^{bcd}	11.9^{bc}	12.3^{cd}	11.9^{cde}
R_0	**S0**	13^{de}	15.6^{ab}	9.3^{d}	12.1^{b}	11.1^{de}	13.8^{b}
	S15	12.9^{def}	15.7^{a}	9.3^{d}	11.7^{bc}	11.2^{cde}	13.7^{b}
	S30	14^{bcd}	12.4^{defg}	11.1^{bc}	11.4^{bc}	12.5^{bc}	11.9^{cde}
$LSD_{0.05}$		**1.6046**		**1.5963**		**1.3135**	
CV(%)		**7.12**		**8.34**		**6.31**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.2.3. Nodule Volume Per Plant

Nodule volume is one of the parameters in assessing the performance of nodules in accordance with their ability to fix atmospheric nitrogen. In Appendix Table 1, the nodule volume which were obtained from both locations and mean values were significantly influenced by the main effect of inoculation, S and Zn and the two way interaction of S and Zn at $P \leq 0.05$. At Tsion and mean value, the two way interaction of inoculation with S and Zn were found significant.

Moreover, this trait at both locations and its mean value over locations were significantly ($P \leq 0.05$) influenced by the three way interaction (Table 3). At both locations, the highest (0.97 and 1.6 ml plant^{-1}) and lowest (0.38 and 0.6 ml plant^{-1}) nodule volume was observed when *Rhizobium* inoculation integrated with 15 kg S ha^{-1} and 1.5 kg Zn ha^{-1}and combined application of *Rhizobium* inoculation and 1.5 kg Zn ha^{-1}, respectively. Indicating S application increase nodule volume (Table 3).

The highest mean value of nodule volume (1.3 ml plant^{-1}) over locations was obtained from combined application of *Rhizobium* inoculation with 15 kg S ha^{-1}and 1.5 kg Zn ha^{-1} which resulted in 116.7% increase over the control check (Table 13). This increment in nodule volume might have been because of synergistic effects between *Rhizobium* inoculation, S and Zn. Similar to the present study, Habtegebrial *et al*. (2007), Alemu (2009), Varin *et al*. (2010) and Workneh *et al*. (2012) have also observed *Rhizobium* inoculation, S and Zn application increase nodule volume of faba bean, fenugreek, white clover and soybean plants, respectively.

Table 3. Nodule volume of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Nodule volume (ml plant^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	0.7^{bc}	0.38^{e}	1.1^{bc}	0.6^{g}	0.9^{bc}	0.5^{f}
	S15	0.52^{d}	0.97^{a}	0.8^{def}	1.6^{a}	0.7^{de}	1.3^{a}
	S30	0.79^{b}	0.67^{c}	1.2^{b}	1.1^{bcd}	1^{b}	0.9^{bc}
R_0	**S0**	0.48^{de}	0.65^{c}	0.7^{fg}	1^{fg}	0.6^{ef}	0.8^{cd}
	S15	0.5^{d}	0.72^{bc}	0.7^{efg}	1.1^{bc}	0.6^{ef}	0.9^{bc}
	S30	0.75^{bc}	0.64^{c}	1.2^{bc}	1^{bcd}	1^{bc}	0.8^{cd}
$LSD_{0.05}$		**0.11**		**0.238**		**0.15**	
CV(%)		**10.19**		**14**		**10.75**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.2.4. Nodule Dry Weight per Plant

In Appendix Table 1, nodule dry weight data which were obtained from both locations and its mean value was significantly affected by the main effects of inoculation and S and its two way interaction between them at $P \leq 0.01$. Similarly, the main effect of Zn was found significant at Tsion and Denzaz site at $P \leq 0.01$. The analysis of variance also showed that the two way interaction of S with Zn was significantly influenced this trait at both locations and mean value combined over locations at $P \leq 0.01$ The interaction of the three factors also significantly ($P \leq 0.01$) influenced the nodule dry weight at both sites as well as their mean value over locations (Table 4).

At Tsion site, the highest (58.7 mg plant^{-1}) and lowest (29.3 mg plant^{-1}) nodule dry weight values were obtained in response to *Rhizobium* inoculation when integrated with 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} and *Rhizobium* inoculation alone, respectively. At Denzaz site, the highest (46.7 mg plant^{-1}) and lowest (14.7 mg plant^{-1}) nodule dry weight were obtained from *Rhizobium* inoculation when integrated 15 kg S ha^{-1} and application of 1.5 kg Zn ha^{-1} alone, respectively.

The highest (48.5 mg plant^{-1}) mean value of nodule dry weight combined over locations was found when *Rhizobium* inoculation was integrated with 15 kg S ha^{-1} which resulted in 22.2% increase over the control check (Table 4). This is probably due to the positive role of S in promoting nodulation and enhancement of photosynthesis in plants. Consistent with this idea, Scherer (2008) has noted that root and nodule development of legumes root is promoted by S fertilization. Scherer and Lange (1996) also reported that S deficiency decreased N demand, which in turn decreased the number and mass of nodules. In contrast, an increase in N demand resulted in higher number and mass of nodules. Similarly, different authors reported that the dry weight of nodules increased with *Rhizobium* inoculation (Kantar *et al*., 2003; Habtegebrial *et al*., 2007; Rokhzadi and Toashih, 2011; Abdalla *et al*., 2011; Birhanu and Pant, 2012; Workneh *et al*., 2012; Jay *et al*., 2012; Srinivasulu *et al*., 2015; Sharifi, 2016) and S application (Yadav, 2011; Rakesh *et al*., 2012; Sipai *et al*., 2016).

Table 4. Nodule dry weight of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Nodule dry weight(mg plant^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	29.3^{f}	44^{d}	16^{hi}	31.3^{b}	22.7^{g}	37.7^{c}
	S15	50.3^{bc}	36.3^{e}	46.7^{a}	22^{eg}	48.5^{a}	29.2^{ef}
	S30	37.3^{e}	58.7^{a}	26^{def}	30.7^{bc}	31.7^{de}	44.7^{b}
R$_0$	**S0**	52^{b}	50.3^{bc}	27.3^{bcd}	14.7^{i}	39.7^{c}	32.5^{d}
	S15	47^{cd}	37.3^{e}	26.7^{cde}	19.3^{gh}	36.8^{c}	28.3^{f}
	S30	39^{e}	47.7^{bcd}	22.7^{efg}	28^{bcd}	30.8^{def}	37.8^{c}
LSD$_{0.05}$		**4.38**		**4.57**		**3.03**	
CV(%)		**5.86**		**10.39**		**5.11**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=R*hizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.2.5. Effective Nodule

Many authors have reported that legume nodules having dark pink or red colors due to presence of leghemoglobin are an indication for effectiveness of the rhizobial strains used, which is well correlated with nitrogen fixation (Adjei and Chambeiss, 2002; Butler and Evers, 2004). The effectiveness of nodules in its ability to fix atmospheric nitrogen in response to inoculation, S and Zn was assessed using nodule color. In Appendix Table 2, neither the main effect of all factors and nor the two way interaction between them was found significant. The result also indicated that all levels of inoculation, S and Zn were invariably slightly dark red.

The three way interaction between inoculations, S and Zn was presented in Table 5. Nodule color was found to range from pink to slightly dark red. The color observed in the inoculated and un-inoculated plots was comparable to each other indicating the non-effectiveness of inoculated rhizobia over the native rhizobia.

Table 5. Effectiveness of nodules of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Effectiveness of nodules**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	2.6^{ab}	2.6^{ab}	2.6^{abc}	2.8^{ab}	2.6^{abcd}	2.7^{abc}
	S15	2.7^{ab}	2.4^{c}	2.9^{ab}	2.7^{abc}	2.8^{ab}	2.5^{cd}
	S30	2.6^{abc}	2.7^{ab}	2.5^{bc}	2.7^{abc}	2.6^{bcd}	2.7^{abcd}
R_0	**S0**	2.8^{a}	2.7^{ab}	2.8^{ab}	2.7^{abc}	2.8^{a}	2.7^{abcd}
	S15	2.5^{bc}	2.7^{ab}	2.4^{c}	2.8^{ab}	2.5^{d}	2.8^{abc}
	S30	2.6^{ab}	2.7^{ab}	2.6^{abc}	2.9^{a}	2.6^{abcd}	2.8^{a}
$LSD_{0.05}$		**0.1967**		**0.325**		**0.23**	
CV(%)		**4.41**		**7.12**		**5.1**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.2.6. Nodulation Rating

Nodulation rating was significantly influenced by the main effect inoculation and S at both locations (Appendix Table 2). The two way interaction between inoculation and Zn was found significant at both locations and mean value combined over location. The two way interaction between inoculation and S also found significant ($P \leq 0.05$) at Denzaz and mean value combined over locations at $P \leq 0.01$ (Appendix Table 2). Appendix Table 2 also revealed that the two way interaction of S and Zn was found significant at Tsion and mean value combined over locations. Moreover, this trait and its mean value combined over locations were significantly influenced by the three way interaction of the three factors at $P \leq 0.05$ (Table 6).

At Tsion site, the highest (6.5) and lowest (3.5) nodulation rating were recorded with combined application of *Rhizobium* inoculation with 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} and 30 kg S ha^{-1} alone, respectively (Table 6). At Denzaz, the highest (7) and lowest (2.7) nodulation rating was recorded from *Rhizobium* inoculation when integrated with 30 kg S ha^{-1} and combined application of 15 kg S ha^{-1} with 1.5 kg Zn ha^{-1}, respectively. The present study also found that, the highest (6.7) mean nodulation rating over locations was obtained with *Rhizobium* inoculation when integrated with 30 kg S ha^{-1} which resulted in 86.1% increase over the control check (Table 16). This is probably due to the availability of optimal level of nutrients for the production of effective and large nodules on the tap root system. In consistent with this suggestion, Jennings (2004) reported that effective nitrogen fixing are often found when the nodules are red and found in the primary root. In general, the result revealed that regardless of S and Zn application rates, the superior nodulation rating of chickpea was gained from *Rhizobium* inoculation over un-inoculated treatment (Table 6).

Table 6. Nodulation rating of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Nodulation Rating**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	6^{b}	4.6^{d}	5.7^{c}	4.6^{d}	5.9^{c}	4.6^{d}
	S15	6^{b}	5.4^{c}	5.8^{c}	6.4^{b}	5.9^{c}	5.9^{c}
	S30	6.4^{ab}	6.5^{a}	7^{a}	6.4^{b}	6.7^{a}	6.5^{b}
R_0	**S0**	3.8^{e}	3.8^{e}	3.4^{f}	4.6^{d}	3.6^{g}	4.2^{f}
	S15	4.3^{d}	3.6^{e}	3.8^{e}	2.7^{g}	4^{f}	3.1^{h}
	S30	3.5^{e}	5.9^{b}	3.9^{e}	4.6^{d}	3.7^{g}	5.3^{d}
LSD$_{0.05}$		**0.4406**		**0.2772**		**0.2086**	
CV(%)		**5.22**		**3.33**		**2.49**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV Coefficient of variation

4.3. Growth Parameters

4.3.1. Plant Height

The plant height which was obtained from both locations and its mean values combined over location was significantly affected by the main effect of S application and its interaction with inoculation (at $P \leq 0.01$) (Appendix Table 2 and Table 7). Moreover, this trait and its mean values was significantly influenced by the two way interaction of S and Zn at Denzaz and mean value combined over location. The analysis of variance also showed that the main effect of Zn was found to be significant at Tsion and mean value combined over locations (Table 7). In general, the result revealed that plant height was increased with increasing S rate when the plant was inoculated (only at Denzaz) and fertilized with 1.5 kg Zn ha^{-1} both at Tsion site and mean value combined over locations (Table 7). The highest plant height at both locations and mean value combined over locations were observed with application of 30 kg S ha^{-1}. Sulfur is also a major component of ferreoxin in chloroplast which is relevant for the proper photosynthetic activity (Fukuyama, 2004). Hussain *et al*. (2011) reported that application of 30 kg S ha^{-1} on soybean plants increase plant height by 14% compared with the control. At Tsion and mean value combined over locations, the highest plant height was observed with application of 1.5 kg Zn ha^{-1} (Table 7). This might be attributed to the fact that Zn can activate certain enzymes which are responsible for cell division and elongation which could lead to increased plant height (Nadergoli *et al*., 2011). At Denzaz site rhizobium inoculation resulted in highest plant height while at Denzaz the highest plant height was obtained from un inoculated treatment. Similarly, previous research also confirmed that plant height was increased with S application (Nasreen and Farid, 2006; Ram and Katiyar, 2013; Sipai *et al*., 2016) and Zn application (Nadergoli *et al*., 2011; Dashadi *et al*., 2013; Usman *et al*., 2014; Kayan *et al*., 2015).

Table 7. Effect of *Rhizobium*, S and Zn fertilizer rates on plant height of chickpea

	PH		
	Tsion	**Denzaz**	**Mean**
	Inoculation		
R_0	39.6^a	32.7^b	36.1
R_1	38.9^b	33.3^a	36.1
LSD(0.05)	**0.44**	**0.41**	**ns**
	Levels of S		
S0	38.2^b	32.9^b	35.6^c
S15	39.6^a	32^c	35.8^b
S30	39.9^a	34.1^a	37^a
LSD(0.05)	**53.4**	**0.51**	**0.58**
	Levels of Zn		
Zn0	38.8^a	32.9	36.2^a
Zn1.5	39.6^b	33.1	36^b
LSD(0.05)	**0.44**	**ns**	**0.31**
I*S	**	**	**
I*Zn	ns	ns	ns
S*Zn	ns	**	**
I*S*Zn	ns	ns	ns
CV(%)	**1.61**	**1.82**	**1.25**

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated , S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.3.2. Root Length

The main effect of inoculation and S were significantly ($P \leq 0.05$) affected the root length at both location and mean value combined over location (Appendix Table 3). The two way interaction of inoculation and S was found to be significant at Denzaz and Mean value combined over location. At Tsion and mean value combined over location, the two way interaction between inoculation and Zn was significantly influenced this trait. The analysis of variance also showed that the two way interaction between S and Zn was significantly influenced this trait at Tsion and Denzaz. Moreover, this trait was significantly influenced by the three way interaction. At Tsion site, the highest (21.1 cm) and lowest (17 cm) root lengths were obtained from the combination of *Rhizobium,* 15 kg S and 1.5 kg Zn ha^{-1} and from the combination of *Rhizobium,* 30 kg S and 1.5 kg Zn ha^{-1} (Table 8). Previous finding also confirmed that *Rhizobium* inoculation (Bhuiyan *et al*., 2008; Ali *et al*., 2008; Nishita and Joshi, 2010), S (Varin *et al*., 2010; Khan and Mazid, 2011) and Zn application (Khan, 1998; Yohannes *et al*., 2015) significantly increased root length. Moreover, regardless of inoculation and Zn application, application of S up to 15 kg S ha^{-1} resulted in increased root length. Beyond this rate further increase in root length was not observed. This may be mainly because of the development of acidity environment in immediate vicinity of root zone due to high S rate application and this may retard root growth (Rengel, 2003). Similarly, Zhao *et al*. (2008) observed that basal application of 30 mg kg^{-1} elemental S increase root length compared with the control check on soybean but it decreases root length compared with 15 mg kg^{-1} S.

Table 8. Root length of chickpea as affected by *Rhizobium*, S and Zn fertilizer rates

Treatment		**Root length (cm)**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
	S0	19.9^{ab}	17.6^{de}	16.3^{cd}	17.6^{ab}	18.1^{bc}	17.6^{cdef}
$\mathbf{R_1}$	**S15**	19.7^{ab}	21.1^{a}	18.0^{a}	17.3^{abc}	18.8^{ab}	19.2^{a}
	S30	19.1^{bc}	17^{e}	16.4^{bcd}	16.1^{cd}	17.7^{cde}	16.5^{fg}
	S0	17.4^{de}	18.1^{cde}	16.5^{bcd}	16.9^{abcd}	16.9^{defg}	17.5^{cdef}
$\mathbf{R_0}$	**S15**	18.7^{bcd}	19.2^{bc}	15.8^{de}	14.2^{f}	17.2^{cdef}	16.7^{efg}
	S30	17.5^{de}	18.5^{bcde}	14.5^{ef}	17.0^{abc}	16^{g}	17.8^{bcd}
LSD(0.05)		**1.517**		**1.259**		**3.57**	
CV (%)		**4.77**		**4.54**		**1.066**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, I R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, NS = Not significant at $P \leq 0.05$, CV = Coefficient of variation

4.3.3. Shoot Dry Weight

The main effect of S application and its interaction with inoculation and Zn significantly influenced shoot dry weight at Denzaz and mean value combined over locations but not at Tsion at $P \leq 0.05$ (Appendix Table 3). The two way interaction between inoculation with S and Zn also found significant at Denzaz and mean value combined over locations. In general, regardless of the treatments applied, the data showed that shoot dry weight at Tsion was higher than those obtained at Denzaz (Table 9). Higher moisture availability at the time of sowing at Tsion site could result in better germination and ultimately good crop stand and higher shoot dry weight. In general, shoot dry weight exhibited an increasing trend with S application rates when the plant was inoculated and fertilized with 1.5 kg Zn ha^{-1}.

At Denzaz site, significantly the highest (6.5 g plant^{-1}) shoot dry weight was found when 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} was applied in combination. The highest mean value of shoot dry weight over locations (7 g plant^{-1}) was obtained from combined application of 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} which resulted in 40% increase over the control check. This might be due to the fact that Zn activates several enzymes such as auxin which is relevant in plant cell division and elongation (Marschner, 1995; Cakmak *et al*., 1989) and thus, leads to enhance dry matter production of the plants. Hussain *et al*. (2011) reported that application of 30 kg S ha^{-1} on soybean plants increase dry matter yield by 26% compared with the control. Valencino *et al*. (2010) also reported that application of 8 mg Zn pot^{-1} increse shoot dry weight of chickpea by 11.4% over the control. In line with this finding, several authors also reported the positive effect of S and Zn applications in shoot dry weight (Hussain *et al.,* 2011; Singh *et al*., 2012; Banik and Sengupta, 2012; Kesare, 2014).

Table 9. Shoot dry weight of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Shoot dry weight(g plant^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	6.5	8.0	3.9^{e}	4.9^{bc}	5.2^{de}	5.7^{cde}
	S15	7.7	7.0	5^{bc}	4.3^{cde}	6.4^{abc}	6.4^{bc}
	S30	7.3	7.2	6.4^{a}	4.7^{bcd}	6.8^{ab}	5.9^{cde}
R_0	**S0**	6.3	7.4	3.7^{ef}	4.9^{bc}	5^{e}	6.1^{cd}
	S15	6.4	8.1	4de	3.1^{f}	5.2^{de}	5.6^{cde}
	S30	7.4	7.6	5.2^{b}	6.5^{a}	6.3^{bc}	7^{a}
LSD0.05		**NS**		**0.756**		**0.811**	
CV(%)		**9.69**		**9.49**		**7.99**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, NS = Not significant at $P \leq 0.05$, CV = Coefficient of variation

4.3.4. Root Dry Weight

The main effect of S application and its interaction with Zn was significantly influenced root dry weight at both sites at $P \leq 0.05$. The result also found that at Tsion and on mean value of this trait over locations was significantly influenced by the main effect of inoculation and its interaction with Zn (Appendix Table 3). At Denzaz and mean value of this trait also influenced by the main effect Zn and its interaction with inoculation and S (Appendix Table 3). Moreover, the three way interaction were significantly ($P \leq 0.05$) affected the root dry weight at both locations and their mean value over locations. At Tsion site, the highest root dry weight (0.71 g plant^{-1}) was recorded with combined application of 15 kg S ha^{-1} and 1.5 kg Zn ha^{-1} under un inoculated condition while the highest value (0.54 g plant^{-1}) at Denzaz site were found from 30 kg S ha^{-1} applied with 1.5 kg Zn ha^{-1} under un inoculated condition (Table 10). Combined application of *Rhizobium* inoculation and 30 kg S ha^{-1} resulted in the maximum mean root dry weight (0.59 g plant^{-1}). Similarly, root dry weight increase due to *Rhizobium* inoculation (Abdalla *et al*., 2011; Workneh *et al*., 2012; Jay *et al*., 2012), and S application (Besharati and Rastin, 1999; Zhao *et al*., 2008; Varin *et al*., 2010) have been reported.

Table 10. Root dry weight of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Root dry weight (g plant^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
	S0	0.51^{de}	0.63^{b}	0.39^{e}	0.49^{ab}	0.45^{ef}	0.56^{ab}
R_1	**S15**	0.67^{ab}	0.58^{c}	0.45^{bc}	0.41^{de}	0.56^{ab}	0.5^{c}
	S30	0.67^{ab}	0.5^{ef}	0.51^{ab}	0.4^{e}	0.59^{a}	0.45^{ef}
	S0	0.55^{cd}	0.55^{cde}	0.38^{e}	0.53^{a}	0.47^{de}	0.56^{ab}
R_0	**S15**	0.46^{f}	0.71^{a}	0.39^{e}	0.3^{f}	0.43^{f}	0.5^{c}
	S30	0.54^{cd}	0.58^{cd}	0.43^{de}	0.54^{a}	0.49^{cd}	0.57^{ab}
$LSD_{0.05}$		**0.0477**		**0.0456**		**0.0308**	
CV(%)		**4.84**		**6.23**		**3.57**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.4. Yield Related Traits

4.4.1. Number of Primary Branches

Number of primary branches was responded significantly to the main effect of S application and its interaction with inoculation and Zn at both locations and at Denzaz site respectively at $P \leq 0.05$ (Appendix Table 4). The analysis of variance also showed that the main effect of Zn application (at Tsion site) and its interaction with inoculation was significantly influenced this trait at both locations and mean value combined over locations. Moreover, this trait by location and its mean value over locations were significantly influenced by the three way interactions at $P \leq 0.05$ (Table 11).

Even though it is not consistent, this observation suggests number of primary branches exhibited an increased with increasing the rate of S and Zn (Table 11). At Tsion site, the highest (4.5) and lowest (3.4) primary branches were obtained from sole application of 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1}, respectively. The highest (3.4) and lowest (2.1) number of primary branches at Denzaz site was obtained in response to combined application of *Rhizobium* inoculation and 30 kg S ha^{-1} as well as with sole application of S at 15 kg ha^{-1} and with the control treatment, respectively (Table 11).

The highest mean value of primary branches over locations (3.8) was obtained from combined application of *Rhizobium* inoculation and 30 kg S ha^{-1} which resulted in 31.03% increase over the control check (Table 11). The increase in primary branches due to *Rhizobial* inoculation was explained by the increasing supply of N through BNF. Application of S has vital role in the primary and secondary metabolism as it is a constituent of various organic compounds (Hitsuda *et al*., 2004; Naeve and Shibles, 2005). Similarly, the number of primary branches increased due to *Rhizobium* inoculation and S application (Sharma and Room, 1997; Togay *et al*., 2008; Namvar *et al*., 2011; Ram and Katiyar, 2013; Kesare, 2014; Jadeja *et al*., 2016; Das *et al*., 2016) have been reported.

Table 11. Number of primary branches of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Number of primary branches (no plant^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	3.5^{e}	3.8^{bcde}	2.7^{cdef}	2.5^{f}	3^{ef}	3.1^{def}
	S15	4.3^{abc}	4.2^{abc}	2.5^{f}	2.7^{def}	3.2^{cde}	3.5^{b}
	S30	3.8^{bcde}	3.7^{de}	3.4^{a}	2.9^{bcde}	3.8^{a}	3.4^{bc}
R$_0$	**S0**	3.8^{cde}	3.4^{e}	2.1^{g}	3^{bcd}	2.9^{f}	3.4^{bc}
	S15	3.9^{bcd}	3.7^{de}	3.4^{a}	2.6^{ef}	3.4^{bc}	3.3^{bcd}
	S30	4.5^{a}	3.5^{e}	3^{bc}	3.1^{b}	3.3^{bcd}	3.7^{a}
LSD$_{0.05}$		**0.434**		**0.32**		**0.25**	
CV(%)		**6.62**		**6.7**		**4.4**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.4.2. Number of Pods Per plant

Number of pods per plant at both locations was significantly affected by the main effect of S and Zn at $P \leq 0.01$ (Appendix Table 4). At Tsion and Denzaz site, the two way interaction between inoculation with S and Zn was found significant ($P \leq 0.05$) respectively. More over at

Denzaz and mean value combined over locations, the two way interaction S and Zn was found significant. The analysis of variance also revealed that this trait and its mean value over locations were significantly influenced by three way interactions (Table 12).

At Tsion site, significantly the highest number of pod (51.3) was obtained from combined application of *Rhizobium* inoculation with 15 kg S ha^{-1} and 1.5 kg Zn ha^{-1}. But it is the same as combined application of 30 kg and 1.5 kg Zn ha^{-1}. While at Denzaz site the highest value (47.7) was found from combined application of *Rhizobium* inoculation with 30 kg S ha^{-1}. Regardless of locations, the lowest number of pod was obtained from *Rhizobium* inoculation alone. The combined analysis over locations indicated that the highest (48.3) mean number of pod was obtained from combined application of *Rhizobium* inoculation and 30 kg S ha^{-1}. In general, the result demonstrated that number of pod per plant increased with S application rate under inoculated condition with 1.5 kg Zn ha^{-1}. But the trend is not consistent. The increase of number in pods per plant with applications of Zn might be due to the positive effect of Zn on formation of stamens and pollens which could increase number of pods produced in the plant (Usman *et al*., 2014). S plays many important roles in the growth and development of plants including chlorophyll and nitrogenize formation, promotes nodule formation and enzyme activation (Fageria, 2009). Similarly, El-Kadar and Mona (2013) reported that combined application of S and Zn increase pods number by 19.7% over the control. Other researchers (Nasreen and Farid, 2006; Kanase *et al.,* 2006; Togay *et al*., 2008; Zhao *et al*., 2008; Hussain *et al*., 2011; Nasri *et al*., 2011; Najar *et al*., 2011; Namvar *et al*., 2011; Ram and Katiyar, 2013; Kesare, 2014; Kayan *et al*., 2015; Das *et al*., 2016; Jadeja *et al*., 2016) also reported that number of pod increased with *Rhizobium* inoculation, S and Zn application.

Table 12. Number of pods per plant of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Number of pods per plant (no plant^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	S0	42.9^{g}	46.5^{de}	31.4^{f}	33.3^{def}	37.2^{g}	39.9^{ef}
	S15	49.1^{bc}	51.3^{a}	34.9^{cde}	34.8^{cde}	42^{cd}	43.1^{bc}
	S30	40^{bc}	50.4^{ab}	47.7^{a}	33^{def}	48.3^{a}	41.7^{bc}
R_0	S0	48.1^{cd}	48.4^{c}	32.1^{ef}	34.6^{cde}	40.1^{def}	41.5^{cde}
	S15	44.4^{fg}	45.6^{ef}	35.4^{cd}	33.1^{def}	39.9^{ef}	39.3^{f}
	S30	48.2^{cd}	51^{a}	38.6^{b}	37.5^{bc}	43.4^{bc}	44.2^{b}
$LSD_{0.05}$		**1.8117**		**3.12**		**1.98**	
CV(%)		**2.22**		**5.19**		**2.79**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.4.3. Number of Seeds Per Pod

Number of seeds per pod exhibited a significant response to the main effect of S application and its two way interaction with Zn at Denzaz ($P \leq 0.05$) (Appendix Table 5). At Tsion site only the two way interaction between S and Zn was significantly influenced this trait (Appendix Table 5).

The analysis of variance also revealed that the three way interaction significantly ($P\leq 0.05$) affected number of seed at Denzaz site and its mean value over locations (Table 13). In general, the non-significant and lower number of seeds per pod at Tsion than Denzaz was justified by the emergence of pod borer during pod setting period of the crop. At Denzaz site, highest number of seed (1.4) was recorded with combined application of 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1}. The highest mean value of number of seeds per pod over locations (1.3) was recorded with combined application of *rhizobium* inoculation with 30 kg S and 1.5 kg Zn ha^{-1} which resulted in 8.3% increase over the control check. This treatment combination was found statistically as par with combined applicationof 30 kg S and 1.5 kg Zn ha^{-1}. This could be due to the fact that sulfur deficiency causes significant reduction of leaf size and photosynthetic materials and resulted in reduction seed number (Hitsuda *et al*., 2004). *Rhizobium* inoculation provides adequate supply of N for plant and resulted in increased chlorophyll synthesis and photosynthetic products. The result of the present study was in conformity with Shivakumer (2001), Boem *et al*. (2007), Kanase *et al*. (2006), Zhao *et al*. (2008), Togay *et al*. (2008), Nasri *et al*. (2011), Ram and

Katiyar (2013), Kesare (2014), Kayan *et al*. (2015), Sipai *et al*. (2016), Sharifi (2016) and Das *et al*. (2016) who have reported that the role of *Rhizobium* inoculation, S and Zn in increasing seed per pod.

Table 13. Number of seeds per pod of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Number of seed per pod (no pod^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	1.2	1.1	1.3^{ab}	1.3^{abc}	1.2^{abc}	1.2^{abcde}
	S15	1.2	1.2	1.1^{d}	1.2^{cd}	1.2^{de}	1.2^{bcde}
	S30	1.1	1.3	1.3^{ab}	1.3^{abc}	1.2^{bcde}	1.3^{a}
R$_0$	**S0**	1.2	1.2	1.3^{ab}	1.3^{abc}	1.2^{abc}	1.2^{bcde}
	S15	1.3	1.2	1.2^{bcd}	1.1^{d}	1.2^{abcd}	1.2^{cde}
	S30	1.2	1.3	1.1^{d}	1.4^{a}	1.2^{b}	1.3^{a}
LSD$_{0.05}$		**NS**		**0.1059**		**0.0955**	
CV(%)		**5.99**		**5.08**		**4.61**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, NS = Not significant at $P \leq 0.05$, CV = Coefficient of variation

4.4.4. Hundred Seed Weight

The main effect of S affected hundred seed weight at Tsion and Denzaz site ($P \leq 0.05$). At Tsion and mean value, the main effect of inoculation and Zn was found significant ($P \leq 0.01$). The result also indicated that the two way interaction between Zn with inoculation and S was significantly influenced this trait ($P \leq 0.05$) (Appendix Table 5). Moreover, this trait was significantly ($P \leq 0.05$) affected by the three way interactions (Table 14).

At Tsion site, the highest (29.9 g) and lowest (28.2 g) hundred seed weight were obtained from the combined application of *rhizobium* inoculation with 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} as well as sole applicationof 1.5 kg Zn ha^{-1} and *Rhizobium* inoculation alone, respectively. At Denzaz site, the highest (29.6 g) was found from combined application of *Rhizobium* inoculation and 1.5 kg Zn ha^{-1}. At this location the lowest (28.1) was obtaine in response to sole application of *Rhizobium* inoculation and sole application of 30 kg S ha^{-1}. Mean hundred seed weight over locations varied from 28.2 g with *Rhizobium* inoculation alone to 29.41 g with combined application of *rhizobium* inoculation with 30 kg S ha^{-1} and 1.5 kg Zn ha^{-1} application. This increase in 100 grains weight due to *rhizobium* inoculation may be due to the more delivery of nitrogen by biological N_2 fixation (Aslam *et al*., 2010). Zn application also had a pivotal role on crop growth, involving in photosynthesis, respiration and nitrogen metabolism-protein synthesis. The assimilated photosynthates are translocated from vegetative plant parts to the seed, thus, considerably enhance seed weight (Kakiuchi and Kobata, 2008). Better growth and development of crop plants due to S supply and nitrogen uptake might have increased the supply of assimilates to seed, which ultimately gained more weight. Similarly, different authors observed that the importance of *Rhizobium* inoculation, S and Zn in increasing hundred seed weight (Nasreen and Farid, 2006; Namvar *et al.,* 2011; Nasri *et al*., 2011; Sipai *et al*., 2016; Jadeja *et al*., 2016; Das *et al*., 2016).

Table 14. Hundred seed weight of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Hundred seed weight (g 100 seed^{-1})**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	28.2^{e}	28.7^{de}	28.1^{e}	29.6^{a}	28.2^{b}	29.1^{ab}
	S15	28.5^{de}	28.4^{e}	28.4^{cde}	29^{abc}	28.5^{ab}	28.7^{ab}
	S30	29.5^{ab}	29.9^{a}	29.2^{ab}	28.9^{bcd}	29.3^{a}	29.41^{a}
R_0	**S0**	29.4^{ab}	29.9^{a}	29.3^{ab}	28.3^{de}	29.3^{a}	29.1^{ab}
	S15	28.9^{cd}	28.5^{de}	28.1^{e}	29^{abc}	28.5^{ab}	28.8^{ab}
	S30	29.3^{bc}	29.5^{ab}	29.5^{ab}	29.3^{ab}	29.4^{a}	29.4^{a}
LSD$_{0.05}$		**0.492**		**0.71**		**0.38**	
CV(%)		**0.99**		**1.45**		**0.78**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.5. Crop Phenology

4.5.1. Days to 50% Flowering

Reproduction is one of the most important events during the life cycle of higher plants. Several environmental factors such as drought (Sharma and Ashok, 2009), salinity (Bram and Quinn, 2013) and micronutrient stress (Pandey, 2010) affect the normal process of reproduction. Among micronutrient stress that limits reproduction, Zn deficiency has predominant effect on flower initiation and development. Plants exposed to Zn deficiency show delayed flowering, premature bud abscission, reduced seed set and seed yield. Days to 50% flowering was affected significantly ($P \leq 0.05$) by the main effect of inoculation and Zn at Denzaz and S at Tsion site respectively. The two way interaction of inoculation with S with Zn was also found significant at Tsion and Denzaz sites, respectively (Appendix Table 5).

The three way interaction effect of *rhizobium* inoculation, S and Zn was found to be statistically significant ($P \leq 0.05$) at Tsion and Denzaz sites (Table 15). At Tsion site, the longest (52 days) and shortest (47 days) days to 50% flowering were observed with *rhizobium* inoculation alone and sole application of 15 kg S ha^{-1} as well as with interaction between *Rhizobium* inoculation and Zn application at 1.5 kg ha^{-1}, respectively. At Denzaz site, the longest and shortest dates to 50% flowering was found to be 54 and 49 days due to sole application of 15 kg S ha^{-1}and *rhizobium* inoculation plus 1.5 kg Zn ha^{-1} as well, respectively. The highest and the lowest day to 50% flowering at Denzaz site was somewhat elongated than those found at Tsion site. This is probably due to supplementation of the crop with irrigation at Denzaz site and this might lead to elongated period of vegetative growth of chickpea (Rajin *et al*., 2003).

Table 15. Days to 50% flowering of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Days to 50% flowering (no)**				**Mean**	
		Tsion		**Denzaz**			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
	S0	52^{a}	47^{ab}	51^{ab}	49^{b}	51	48
R$_1$	**S15**	50^{ab}	49^{ab}	50^{ab}	52^{ab}	50	51
	S30	50^{ab}	49^{ab}	53^{ab}	52^{ab}	52	51
	S0	50^{ab}	48^{ab}	51^{ab}	52^{ab}	51	50
R$_0$	**S15**	47^{b}	51^{ab}	54^{a}	52^{ab}	51	52
	S30	48^{ab}	50^{ab}	51^{ab}	51^{ab}	49	50
LSD$_{0.05}$		**1.59**		**1.30**		**NS**	
CV(%)		**1.83**		**1.56**		**3.77**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, NS = Not significant at $P \leq 0.05$, CV = Coefficient of variation

4.5.2. Days to Physiological Maturity

Days to physiological maturity was affected by the main effect of S and its two way interaction with inoculation and Zn at both locations and their mean value over locations (Appendix table 5). Moreover, the main effect of Zn was found significant on the mean value of this trait over locations. The analysis of variance also showed that days to physiological maturity and its mean value over locations were significantly influenced by the three way interaction at $P \leq 0.05$ (Table

16). At Tsion site, the longest days to physiological maturity (120.7) was observed with *Rhizobium* inoculation alone. At Denzaz site, the longest days to physiological maturity (108.7) was observed with the control check. The longest days to physiological maturity due to *Rhizobium* inoculation at Tsion site was justified by the fact that *Rhizobium* inoculation enhanced supplies of N through BNF promote vegetative growth. Moreover, the longest days to physiological maturity observed with control check at Denzaz site might be due low fertility status in the study site (Table 1). The present study also indicated that mean value of days to attain physiological maturity over locations were varied from 110 to 114.2 days in response to sole application of 15 kg S ha^{-1} and control check, respectively. This was due to the fact that application of S enhances crop growth and increase nutrient uptake by the crop (Motior *et al*., 2011) and this contributes to reduction of days to physiological maturity.

Table 16. Days to physiological maturity of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Days to physiological maturity (no)**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	120.7^{a}	118^{abc}	106.3^{ab}	106.3^{ab}	113.5^{ab}	112.2^{abcd}
	S15	116^{c}	117^{bc}	105.3^{b}	105.3^{b}	110.7^{cd}	111.2^{bcd}
	S30	116.7^{bc}	115^{c}	106^{ab}	105.3^{b}	111.3^{bcd}	110.2^{cd}
R$_0$	**S0**	119.7^{ab}	118^{abc}	108.7^{a}	107.3^{ab}	114.2^{a}	112.7^{abc}
	S15	114.7^{c}	117^{bc}	105.3^{b}	104.7^{b}	110^{d}	110.8^{cd}
	S30	116.7^{bc}	116.3^{bc}	105.3^{b}	104.7^{b}	111^{bcd}	110.5^{cd}
LSD$_{0.05}$		**3.34**		**3.24**		**2.5**	
CV(%)		**1.69**		**1.81**		**1.33**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.6. Yield and Yield Related Data

4.6.1. Seed Yield

The main effect of *rhizobium* inoculation and Zn application exhibited no significant effect on seed yield at both locations and on mean value combined over locations at $P \leq 0.05$. But the main effect of S application was found significant at $P \leq 0.01$ (Appendix Table 6). In agreement with the present finding, Mondal *et al*. (2005), Islam *et al.* (2011), Banik and Sengupta (2012), Patel *et al*. (2013), Bohra (2014), Das *et al*. (2016), and Jadeja *et al*. (2016) reported that S application increased seed yield of chickpea. It is known that S application enhances chlorophyll concentration, root nodules and dry matter production and this all contribute for yield increment (Erdal *et al.,* 2006). Moreover, acidity produced on oxidation of reduced inorganic sulphur compounds in soil was known to increase the solubility of micronutrients, like iron, zinc and manganese (Vidyalakshmi *et al*., 2009). The analysis of variance also showed that seed yield responded to the two way interaction of S and Zn. Seed yield was also significantly influenced by the three way interaction of inoculation, S and Zn at both locations and its mean combined over locations (Table 17). In general, this result suggests seed was significantly increased with S rates when the plant was inoculated and fertilized with 1.5 kg Zn ha^{-1}. At Tsion site, the highest (2039.8 kg ha^{-1}) seed yield was obtained from the *rhizobium* inoculation integrated with 30 kg S ha^{-1} while the lowest (1693.2 kg ha^{-1}) was from the *rhizobium* inoculation alone.

At Denzaz site, the highest (1515.2 kg ha^{-1}) seed yield was also obtained from *rhizobium* inoculation integrated with 30 kg S ha^{-1} whereas the lowest (1020.5 kg ha^{-1}) was from the control check. Combined over locations, the highest (1777.5 kg ha^{-1}) mean seed yield was obtained from the integrated application of *rhizobium* and 30 kg S ha^{-1} which resulted in 28.02% (389 kg ha^{-1}) yield advantage over the control check (Table 17). The highest yield is probably due to the highest number of pods per plant (Penaloza, 1984; Hardwick, 1988). The present study also revealed that, increasing S and Zn when integrated with *rhizobium* inoculation resulted in seed yield increment until 15 kg S ha^{-1} (Table 17). This was probably due to the impact of S application in increasing the availability of Zn at high pH. Plaster (2013) reported that deficiencies of Zn at higher pH can be corrected by the application of S. The increase in yield might be due to the fact that Zn has beneficial effect in chlorophyll content and helps in the formation of growth hormones and indirectly influence the photosynthesis and reproduction. It also helps in developing the enzyme and vitamins. Sulphur also performs many physiological functions in cystien, methionine and chlorophyll synthesis. This result was in agreement with some previous finding reported on the importance of combined application of S and Zn in increasing seed yield (Chauhan *et al.,* 2013). Similarly, Pable *et al.* (2010) reported that application of 30 kg S and 2.5 kg Zn ha^{-1} in vertisols having deficency in Zn and S significantly increased seed yield of soybean. Moreover, Pable *et al*. (2010) and Pratibha *et al*. (2014) reported the significant interaction of S and Zn on seed yield. Other studies also justified the significant interaction of inoculation (Togay *et al*., 2008; Rokhzadi and Toashih, 2011; Ahmed *et al*., 2010; Namvar and Sharifi, 2011; Namvar *et al.,* 2011), S (Zhao *et al*., 2008; Srinivasarao *et al*., 2008; Islam *et al*., 2011; Kesare, 2014; Zafar *et al*., 2014) and Zn application (Tiwari *et al*., 2006; Srinivasarao *et al*., 2008; Mohammad Reza Haj Seyed Hadi *et al*., 2013; Zafar *et al*., 2014; Sharifi, 2016) on seed yield of chickpea.

Table 17. Seed yield of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Seed yield (kg ha^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	S0	1693.2^{e}	1895.4^{abcd}	1150.3^{ef}	1241.8^{de}	1421.8^{fg}	1568.6^{cde}
	S15	1775.6^{cde}	1903.6^{abcd}	1304.2^{bcde}	1429^{abc}	1539.9^{def}	1666.3^{abcd}
	S30	2039.8^{a}	1797.4^{bcde}	1515.2^{a}	1307.2^{bcde}	1777.5^{a}	1552.3^{cdef}
R$_0$	S0	1756.5^{cde}	1726.6^{de}	1020.5^{f}	1265.6^{cde}	1388.5^{g}	1496.1^{efg}
	S15	1963.5^{ab}	1936.2^{abc}	1307.2^{bcde}	1307.2^{e}	1635.4^{abcd}	1621.7^{abcd}
	S30	1963.5^{ab}	1919.9^{abc}	1405.2^{abcd}	1482.4^{ab}	1684.4^{abc}	1701.2^{ab}
LSD$_{0.05}$		**186.14**		**186.5**		**133.39**	
CV(%)		5.85		8.45		4.94	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation.

4.6.2. Straw Yield

Straw yield which was obtained from Denzaz was significantly influenced by the main effect of S, Zn and two-way interaction of S with inoculations and Zn at $P \leq 0.05$. At Tsion site, the main effect of *rhizobium* inoculation and Zn and the two-way interaction between S with *rhizobium* inoculation and Zn were significant. Moreover, the two-way interaction of S with *rhizobium* inoculation and Zn application were significant on the mean values over location at $P \leq 0.01$ (Appendix Table 6). Straw yield was also significantly influenced by the three-way interaction of inoculation, S and Zn at both locations and its mean combined over locations (Table 18).

At Tsion site, the highest (1398.4 kg ha^{-1}) straw yield was obtained from *rhizobium* inoculation alone while the lowest (1051.2 kg ha^{-1}) was from application of 1.5 kg Zn ha^{-1}. At Denzaz site, the highest straw yield (1491.2 kg ha^{-1}) was obtained from combined application of *rhizobium* inoculation with 1.5 kg Zn ha-1. Combined over locations, the highest (1370.6 kg ha^{-1}) mean straw yield was obtained from combined application of *rhizobium* inoculation and 1.5 kg Zn ha^{-1} which resulted in 27.6% straw yield advantage over the control check. In consistent with the current finding, Das *et al.* (2012) reported that *rhizobium* inoculation with 10 kg $ZnSO_4$ and 25 kg $ZnSO_4$ ha^{-1} resulted in increased straw yield. Similar result were concluded by Sipai *et al*. (2016) and Pable *et al*. (2010) who found positive role of *rhizobium* inoculation and Zn application on straw yield for mungbean and soybean, respectively. Srivastava *et al*. (2006) also reported that the combined application of *rhizobium* inoculation with 30 kg S and 5 kg Zn ha^{-1} increase straw yield of summer green gram.

Table 18. Straw yield of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Straw yield (kg ha^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	1398.4^{a}	1250^{bcd}	1015.4^{e}	1491.2^{a}	1206.9^{de}	1370.6^{a}
	S15	1190.1^{cd}	1239.1^{bcd}	1369.6^{ab}	1194.3^{d}	1279.8^{bcd}	1216.7^{cde}
	S30	1334.4^{ab}	1171^{cd}	1375.5^{abc}	1209.2^{cd}	1355^{a}	1190.1^{de}
R$_0$	**S0**	1160.1^{d}	1051.2^{e}	987.8^{e}	1212.1^{cd}	1074^{f}	1131.7^{ef}
	S15	1269^{bc}	1228.2^{cd}	1206.2^{cd}	1247.7^{abc}	1237.6^{cd}	1238^{abc}
	S30	1334.4^{ab}	1236.4^{bcd}	1390.3^{ab}	1339.9^{bcd}	1362.4^{a}	1288.1^{abc}
LSD$_{0.05}$		**105.38**		**152.39**		**90.87**	
CV(%)		**5**		**7.14**		**4.27**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, I R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.6.3. Harvest Index

Harvest index determines the amount of photosynthates being translocated to the economic parts of plant. Harvest index was influenced significantly ($P \leq 0.05$) due to the main effect of inoculation, Zn and the two way interaction with S at Tsion site. The two way interaction between S and inoculation was also significant ($P \leq 0.05$) in mean value of this trait over locations (Appendix Table 6). Moreover, this trait and its mean value over locations were significantly influenced by three way interactions (Table 19).

At Tsion site, the highest (0.62) and lowest (0.56) harvest Index was measured with control check and *rhizobium* inoculation plus 1.5 kg Zn ha^{-1}, respectively. The highest harvest index observed with control check at Tsion site was justified by the fact that lowest biological yield coupled with lowest uptake of nutrient (N and P) observed with this treatment forced the plant to allocate higher photosynthetic product to the seed. R*hizobium* inoculation plus 1.5 kg Zn ha^{-1} resulted in lower mean harvest index over locations. At Denzaz site, the highest (0.55) and lowest (0.46) harvest Index was recorded with *rhizobium* Inoculation when integrated with 15 kg S and 1.5 kg Zn ha^{-1} and *rhizobium* Inoculation applied with 1.5 kg Zn ha^{-1}, respectively. Previous research finding also indicated the positive role of *rhizobium* inoculation, S and Zn nutrient application in increasing harvest index (Roy *et al*., 1995; Khamparia, 1996; Malik *et al*., 2006; Khorgamy and Farina, 2009; Valenciano *et al*., 2009; Valenciano *et al*., 2011).

Table 19. Harvest Index of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		Harvest index				Mean	
		Tsion		Denzaz			
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	0.6^{ab}	0.56^{c}	0.53^{ab}	0.46^{e}	0.57^{a}	0.51^{b}
	S15	0.61^{ab}	0.6^{ab}	0.49^{cd}	0.55^{a}	0.55^{a}	0.58^{a}
	S30	0.61^{ab}	0.6^{ab}	0.52^{ab}	0.52^{ab}	0.57^{a}	0.56^{a}
R$_0$	**S0**	0.62^{a}	0.6^{ab}	0.51^{bc}	0.52^{bc}	0.56^{a}	0.58^{a}
	S15	0.61^{ab}	0.61^{ab}	0.52^{ab}	0.51^{ab}	0.57^{a}	0.56^{a}
	S30	0.61^{ab}	0.6^{b}	0.5^{bc}	0.52^{ab}	0.57^{a}	0.56^{a}
LSD$_{0.05}$		**0.025**		**0.0283**		**0.02035**	
CV(%)		**2.47**		**8.16**		**2.41**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, R$_1$=*Rhizobium* inoculated, R$_0$=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.7. Nutrient (N and P) Uptake

4.7.1. Total N Uptake

Total N uptake of legume can serve as a good indicator of N$_2$ fixation. Total N uptake which was obtained from both locations and its mean combined over locations was significantly affected by the main effect of S and its two way interaction with Zn at $P \leq 0.05$ (Appendix Table 7). Moreover, the main effect of inoculation and Zn application was found to be significant at Denzaz and mean value combined over locations. The analysis of variance also showed that the two way interaction of inoculation with Zn and S was statistically significant at Denzaz and mean value respectively ($P \leq 0.05$) (Appendix table 7).

The result also found that total N uptake of chickpea was significantly influenced by the three way interaction at $P \leq 0.05$ (Table 20). The highest total N uptake (67.4 kg ha^{-1}) was generally observed in Tsion kebele. This was justified by the fact that highest biological yield also observed in this kebele. The total N uptake of this site varied from 67.4 to 49.8 kg ha^{-1} with

Rhizobium inoculation when integrated with 30 kg S and 1.5 kg Zn ha^{-1} and control check, respectively. At Denzaz site, the highest (52.4 kg N ha^{-1}) and lowest (30.8 kg N ha^{-1}) total N uptake was recorded in response the *rhizobium* inoculation applied with 30 kg S ha^{-1} and at the control check, respectively.

The combined analysis over location indicated that, the highest (58.7 kg ha^{-1}) total N uptake were recorded when *rhizobium* was inoculated with, 30 kg S and 1.5 kg Zn ha^{-1}, which resulted in 45.7% increase over the control check (Table 20). This increment could be attributed to *rhizobium* inoculation helped in biological nitrogen fixation and thus, increase N content in grain and straw. The increase in N uptake as a result of S application may be due to an increment in protein synthesis and enhance photosynthesis (Zhao *et al*., 2008). In the absence of S, amino acids cannot be transformed into proteins, which results in reduced N acquisition (Varin *et al*., 2009). Zn is involved in auxin metabolism like, tryptophane synthesis, tryptamine metabolism, protein synthesis, formation of nucleic acid and helps in utilization of nitrogen as well as phosphorus by plants (Ram and Katiyar, 2013).

Similar to the current findings N uptake increase due to *rhizobium* inoculation, S and Zn application have been reported by several literatures (Zaidi *et al*., 2003; Rokhzadi and Toashih, 2011; 2012; Sharma and Gupta, 1992; Mondal *et al*., 2005; Shamima and Farid, 2006; Srivastava *et al.,* 2006; Togay *et al.,* 2008; Najar *et al*., 2011; Abdalla *et al*., 2011; Hussain *et al*., 2011; Jay *et al*., 2012; EL-Kader and Mona, 2013; Muhammad *et al*., 2013; Kesare, 2014; Yohannes *et al*., 2015; Srinivasulu *et al*., 2015; Das *et al*., 2016; Zerihun *et al*., 2017)

Table 20. Total Nitrogen uptake of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Total Nitrogen Uptake (kg ha^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R$_1$	**S0**	56.6^{def}	53.4^{efg}	35.6^{h}	41.6^{fg}	46.1^{f}	47.5^{f}
	S15	60.4^{bcd}	57.3^{cde}	45.6^{de}	47.4^{cd}	53^{cde}	52.3^{bde}
	S30	58.4^{cd}	67.4^{a}	52.4^{a}	50.1^{ab}	55.4^{bc}	58.7^{a}
R$_0$	**S0**	49.8^{g}	52.3^{fg}	30.8^{i}	39.7^{g}	40.3^{g}	46^{f}
	S15	60^{bcd}	61.5^{bc}	43.6^{ef}	44.1^{ef}	51.8^{e}	52.8^{de}
	S30	61.5^{bc}	64.6^{ab}	48.6^{bc}	51.3^{a}	55^{cd}	58^{ab}
LSD$_{0.05}$		**4.88**		**2.4**		**2.58**	
CV(%)		**4.88**		**3.21**		**2.95**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, I+=*Rhizobium* inoculated, I-= un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.7.2. Total P Uptake

The main effect of S and Zn at both locations and mean value over location significantly influenced the total P uptake at $P \leq 0.01$. Similarly, the main effect of inoculation was found to be statistically significant at Tsion site and mean value at $P \leq 0.01$ (Appendix Table 7). The analysis of variance also showed that, the two way interaction of S with inoculation and Zn application were found to be significantly ($P \leq 0.05$) influenced total P uptake at both location and mean value combined over locations. Moreover, total P uptake was affected by the three way interaction (Table 21). At Tsion site and mean value over locations, the highest and lowest total P uptakes were found due to combined application of 30 kg S with 1.5 kg Zn ha^{-1} and *Rhizobium* inoculation, respectively. But the highest mean value was Statistically as par with 30 kg S ha-1application. At Denzaz site, the highest (10.5 kg ha^{-1}) total P uptake was obtained with sole application of 30 kg S ha^{-1}. The highest mean total P uptake increase by 65.7% over the control check. This could be due to the fact that S application increases P availability in the soil by which enhance the P uptake by plant (Fageria, 2009). This attributed to the fact that oxidation of S produce H_2SO_4 which could solubilize P. Similarly, Kapoor and Mishra (1989) found that the acidity generated on oxidation of pyrite can be coupled to solublization of rock phosphate. Gowda *et al*. (2001) also found that the increase in available P in soil solution is attributed to ion exchange with sulphate-S ion. Previously, seed P uptake increased in response to S application have been reported (Togay *et al*., 2008; Najar *et al*., 2011; Yadav, 2011; Muhammad *et al*., 2013; Kesare, 2014; Das *et al*., 2016; Srinivasulu *et al*., 2015; Zerihun *et al*., 2017). In contrary, Srivastava *et al*. (2006) reported that *Rhizobium* inoculation with 30 kg S and 5 kg Zn increase P total uptake of summer green gram.

Table 21. Total Phosphorus uptake of chickpea as affected by *Rhizobium* inoculation, S and Zn fertilizer rates

Treatment		**Total Phosphorus Uptake (kg ha^{-1})**					
		Tsion		**Denzaz**		**Mean**	
		Zn0	**Zn1.5**	**Zn0**	**Zn1.5**	**Zn0**	**Zn1.5**
R_1	**S0**	8.2^{f}	8.6^{ef}	6.2^{g}	9.7^{abcd}	7.2^{f}	9.1^{d}
	S15	10.8^{cd}	11^{cd}	9.9^{abc}	8.9^{cde}	10.4^{bc}	9.9^{c}
	S30	10.7^{d}	12.5^{b}	9.8^{abcd}	8.9^{def}	10.3^{c}	10.7^{b}
R_0	**S0**	8.4^{f}	9.1^{e}	5.6^{g}	8^{f}	7^{f}	8.6^{e}
	S15	12^{b}	11.3^{c}	8.5^{ef}	9.2^{bcde}	10.2^{bc}	10.2^{bc}
	S30	12.7^{b}	13.2^{a}	10.5^{a}	10^{ab}	11.6^{a}	11.6^{a}
$LSD_{0.05}$		**0.55**		**0.92**		**0.51**	
CV(%)		**3**		**6.21**		**3.09**	

Means with the same letter are not significantly different at P>0.05 level of probability following LSD, I R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, LSD= Least significant difference, CV = Coefficient of variation

4.8. P Harvest Index and P Use Efficiency

In figure 20 and 22 show that the highest P harvest index were recorded with *rhizobium* inoculation and application of 1.5 kg Zn ha^{-1} increase mean P harvest index by 13.3% and 11.7% over the negative control, respectively. Regardless of S application rate, it was increased P harvest index when compared with negative control (Figure 21). The result also demonstrated that at both locations, the highest P use efficiency (77.3% at Tsion site and 163.6% at Denzaz site) were obtained with *Rhizobium* inoculation which resulted in 31% and 45.3% increase over the un-inoculated treatment (Figure 23). Application of 15 kg S ha^{-1} also caused the highest P use efficiency at both locations and mean value over location. This treatment increased P use efficiency by 12.6% over S control.

The increase in P use efficiency due to *Rhizobium* inoculation and S application could be due to the need of high P for ATP synthesis as result of high BNF activity and increase the P availability due to S application. Similarly, Khair *et al*. (2002) reported that *Rhizobium* inoculation increased P uptake efficiency. Ahirwar *et al*. (2016) also indicated that *Rhizobium* inoculation increase agronomic and physiologic P use efficiency by 14.2% and 13.9 over the control in pigeon pea. Ahmad *et al*. (1994) found that NPK application under S deficient condition did not increase the P use efficiency and crop yield in sustainable ways. in contrast to this, Zn application did not affect P use efficiency (Figure 25). This could be attributed to the fact that application of Zn to plants grown in Zn deficient soils is effective in reducing uptake and accumulation of P (and phytate) in plants (Mousavi, 2012).

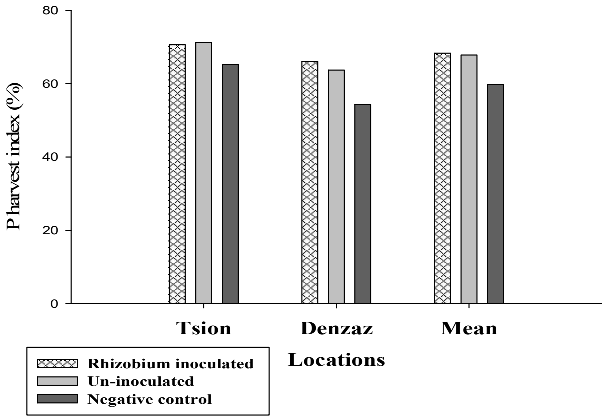

Figure 5. P harvest index as influenced by level of inoculation

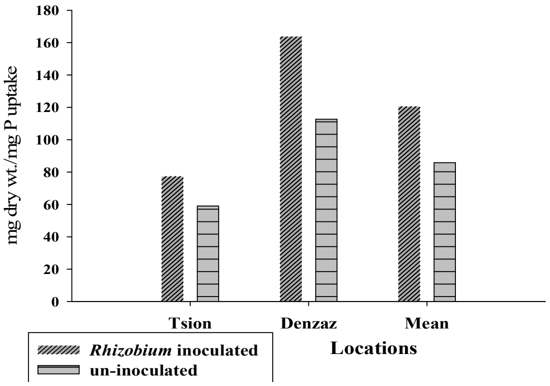

Figure 8. P Use efficiency as influenced by level of Inoculation

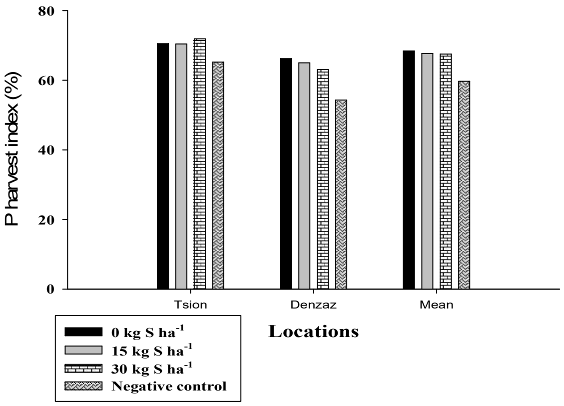

Figure 6. P harvest index as influenced by rate of S application

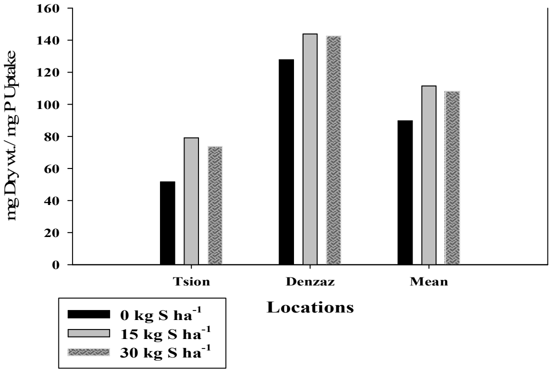

Figure 9. P Use efficiency as influenced by rate of S application

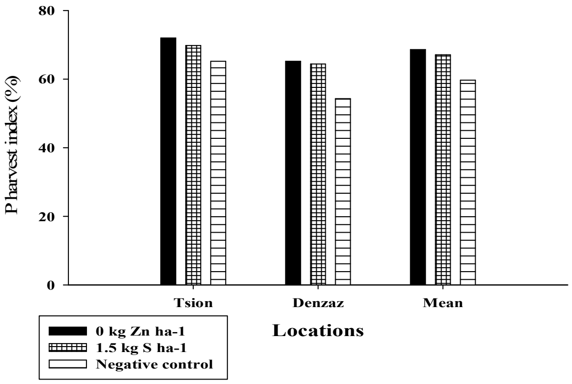

Figure 7. P harvest index as influenced by rate of Zn application

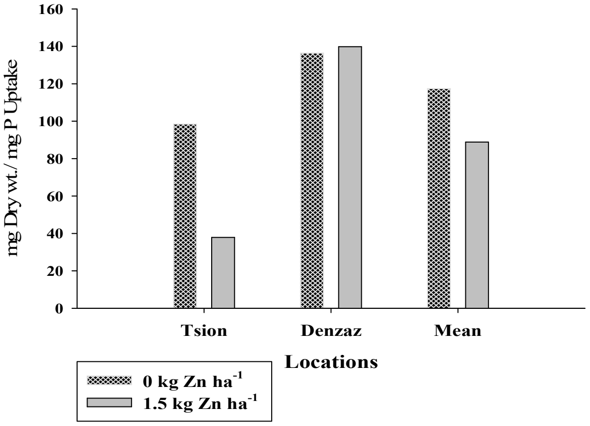

Figure 10. P Use efficiency as influenced by rate of Zn application

4.9. Partial Budget Analysis

It is quite evident from the data presented in Table 22, that the highest mean total gross benefit (38433.4 birr ha^{-1}) and mean net benefit (37069.4 birr ha^{-1}) was obtained when *Rhizobium* applied with 30 kg S ha^{-1}. The next better return was 35485.5 ha^{-1} birr which was obtained from 30 kg S applied together with 1.5 kg Zn ha^{-1}. The lowest mean total gross benefit and mean net benefit of 30050.3 birr ha^{-1} was obtained from the control check and found net benefit penalty of 23.4% (7019.1 birr ha^{-1}).

According to the dominance analysis on mean value over locations, control check, R_1 (*Rhizobium* inoculation alone), R_1S15 (*Rhizobium* inoculation + 15 kg ha^{-1} Sulphur) and $R_1S30Zn1.5$ (*Rhizobium* inoculation + 30 kg ha^{-1} Sulphur + 1.5 kg ha^{-1} Zinc) were dominated by other treatments, hence, eliminated from further economic analysis (Figure 26). The highest MRR (marginal rate of return) of 1941% was obtained from combined application of *Rhizobium* inoculation and 30 kg S ha^{-1} (Table 23). This implies that for 1.00 birr investment in chickpea production, the producer can get 19 birr.

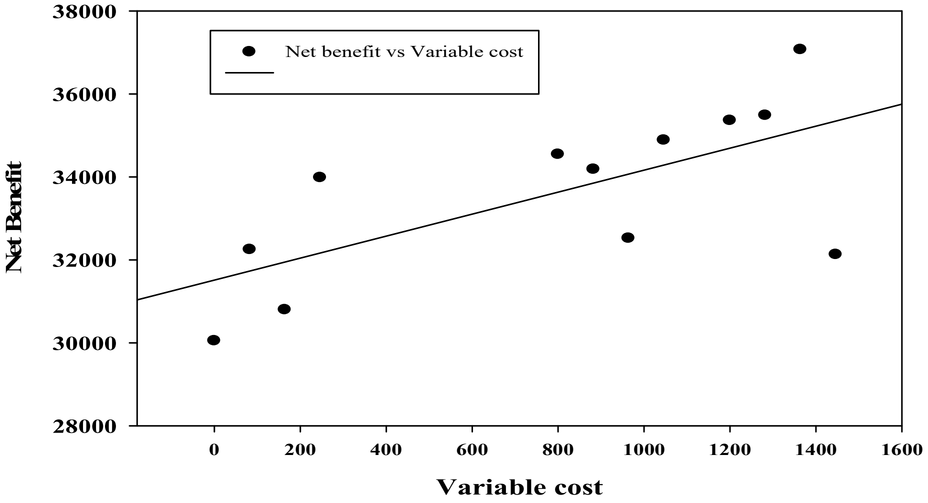

Figure 11. Net benefit Curve

NB=Note- treatments with dots below the line are dominated treatments, hence are not attractive economically.

Table 22. Partial Budget Analysis

Treatment	AGY	ASY	GBG	GBS	TGB	TVC	NB	D
R_1	1279.6	1086.2	28791.5	2172.4	30963.9	164	30799.9	DM
R_1Zn1.5	1411.7	1233.5	31764.2	2467.1	34231.2	246	33984.8	
R_1S15	1385.9	1151.8	31183.0	2303.6	33486.6	964	32522.6	DM
R_1S15Zn1.5	1499.7	1095.0	33742.6	2190.1	35932.6	1046	34886.2	
R_1S30	1599.8	1219.5	35994.4	2439.0	38433.4	1364	37069.4	
R_1S30Zn1.5	1397.1	1071.1	31434.1	2142.2	33576.3	1446	32129.9	DM
Control	1249.7	966.6	28117.1	1933.2	30050.3	0	30050.3	DM
Zn1.5	1346.5	1018.5	30296.0	2037.1	32333.1	82.4	32250.7	
S15	1471.9	1113.8	33116.9	2227.7	35344.5	800	34544.5	
S15Zn1.5	1459.5	1114.2	32839.4	2228.4	35067.8	882.4	34185.4	
S30	1516.0	1226.2	34109.1	2452.3	36561.4	1200	35361.4	
S30Zn1.5	1531.1	1159.3	34449.3	2318.6	36767.9	1282	35485.5	

R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1},GY= Adjusted seed yield ((kg ha^{-1}), ASY= Adjusted straw yield (kg ha^{-1}), GBS=gross benefit from straw (ETB ha^{-1}), GBG=gross benefit from straw (ETB ha^{-1}), TGB=total gross benefit (ETB ha^{-1}), TVC=total cost that vary (ETB ha^{-1}), NB=net benefit (ETB ha^{-1}), D=dominance, DM=dominated.

Table 23. Marginal analysis of undominated treatment

Treatment	NB	TVC	MB	MC	MRR (%)
Zn1.5	32251	82.4	1734.1	0	0
R_1Zn1.5	33985	246.4	559.7	164	1057
S15	34545	800	-359.1	553.6	101.1
S15Zn1.5	34185	882.4	700.81	82.4	-435.8
R_1S15Zn1.5	34886	1046	475.18	164	427.3
S30	35361	1200	124.06	153.6	309.4
S30Zn1.5	35485	1282	1583.9	82.4	150.6
R_1S30	37069	1364	1734.1	81.6	1941

R_1=*Rhizobium* inoculated, R_0=un-inoculated, S0= 0 kg S ha^{-1}, S15= 15 kg S ha^{-1}, S30= 30 kg S ha^{-1}, Zn0= 0 kg Zn ha^{-1}, Zn1.5=1.5 kg Zn ha^{-1}, TVC=total cost that vary (ETB ha^{-1}), NB=net benefit (ETB ha^{-1}), MRR=marginal rate of return.

5. SUMMARY AND CONCLUSIONS

Nitrogen fixation is one of the most important biological process next to photosynthesis on the earth. However, due to nutrient deficiency, the N_2 fixation is impaired and consequently causes low crop productivity in sub-Saharan Africa. In most tropical soils including in Ethiopia N, P, S and Zn are the major limited nutrients for crop production. Ensuring a well-balanced supply of P, S and Zn to the chickpea crop may result in higher seed yield through improving nodule activities and nitrogen fixation. Hence field experiment was initiated to evaluate the effect of Sulphur and Zinc application with *rhizobium* on the performance of chickpea.

Factorial combinations of two levels of inoculation (*rhizobium* inoculation and un-inoculation), three levels of S (0, 15 and 30 kg S ha^{-1}) and two levels of Zn (0 and 1.5 kg Zn ha^{-1}) in RCB design with three replications were used. All the experimental plots were treated with equal amount of 20 kg N ha^{-1} and 20 kg P ha^{-1}.

Composite surface soil samples (0-30 cm depth) were collected before planting from 5 locations from Gonder Zuria Woreda where non responsiveness to *rhizobium* inoculation and P fertilizer application for chickpea has been observed. The soil analysis indicated that the soil is clayey in texture. The pH (H_2O) of the soil ranged between neutral (pH 7.0) and moderately alkaline (pH 7.9). All sites were very high in CEC, The whole sites were low in OM content with a mean value of 1.16%. Total N ranged from very low to low with mean value of 0.052%. Available (Olsen) P of the soil ranged from low to high with a mean value of 7.42 mg kg^{-1}. At all the tested sites, available S ranged between very low and low. All sites had very high and high in exchangeable Ca^{++}/Mg^{++} and K^{+}, respectively. From the micronutrient investigated, only Zn deficiency was observed in the whole five farms. Based on the soil testing result, S and Zn had been considered as the nutrients to be corrected by applying fertilizer along with *Rhizobium* inoculation.

Analysis of variance revealed that the three way interaction of inoculation, S and Zn nutrient application had significant ($P \leq 0.05$) influence on nodule parameters at both locations and the mean value over locations. At Tsion site, the highest nodule number (15.7) was obtained from the combined application of 15 kg S and 1.5 kg Zn ha^{-1} while at Denzaz site, the highest nodule number (15.8) was obtained from the combined application of *Rhizobium* inoculation, 15 kg S and 1.5 kg Zn ha^{-1}. The highest (15.3) mean nodule number over locations was obtained from *Rhizobium* inoculation integrated with 15 kg S and 1.5 kg Zn ha^{-1} which resulted in 37.8% increment over the control check. The highest mean value of nodule volume (1.3 ml plant^{-1}) over locations was obtained from combined application of *Rhizobium* inoculation with 15 kg S ha^{-1}and 1.5 kg Zn ha^{-1} which resulted in 116.7% increase over the control check. The nodule color was found ranging between pink and slightly dark red. The present study also found that, the highest (6.7) mean nodulation rating over locations was obtained with *Rhizobium* inoculation when integrated with 30 kg S ha^{-1} which resulted in 86.1% increase over the control check.

It was observed that, the three way interaction among inoculation, S and Zn significantly affected all growth trait studied except plant height at both locations, shoot dry weight and number ofseeds per pod at Tsion. The results also demonstrate that, the interaction of the three factors significantly affect crop phenology, seed yield, straw yield, N and P uptake of seed and straw at both locations and their mean.

With respect to seed yield, the main effect of S application significantly ($P \leq 0.05$) increase the seed yield of chickpea at both locations and its mean value over location but not found in inoculation and Zn. Likewise, the three way interaction among inoculation, S and Zn application significantly influenced seed yield. The highest (1777.5 kg ha^{-1}) and lowest (1388.5 kg ha^{-1}) mean seed yield combined over location were observed with combined application of *rhizobium* inoculation plus 30 kg S ha^{-1} and control check, respectively. Mean straw yield of chickpea over location also significantly affected by the interaction of three factors. The highest (1370.6 kg ha^{-1}) and lowest (1074 kg ha^{-1}) were obtained from combined application of *rhizobium* inoculation with 1.5 kg Zn ha-1 and from control check, respectively.

The analysis of variance showed that N and P uptake were significantly affected by the three way interaction. At Tsion site, *Rhizobium* when integrated with 30 kg S and 1.5 kg Zn ha^{-1} application resulted in the highest total N uptake (67.4 kg ha^{-1}) while the highest at Denzaz site (52.4 kg ha^{-1}) was obtained in response to *Rhizobium* when integrated with 30 kg S ha^{-1}. Combined analysis over location also found that the highest total N uptake (58.7 kg ha^{-1}) was recorded when *Rhizobium* inoculation, 30 kg S and 1.5 kg Zn ha^{-1} were applied together, which resulted in 45.7% increase over the control check. Similarly, the highest total P uptake at Tsion and mean value over location were found due to combined application of 30 kg S with 1.5 kg

Zn ha^{-1}. At Denzaz site, the highest (10.5 kg ha^{-1}) and lowest (5.6 kg ha^{-1}) total P uptake was obtained when 30 kg S ha^{-1} applied alone and from the control check, respectively.

The partial budget analysis showed that the maximum mean total gross benefit (38433.4 birr ha^{-1}) and mean net benefit (37069.4 birr ha^{-1}) were obtained from *Rhizobium* when integrated with 30 kg S ha^{-1}. The lowest mean total gross benefit and mean net benefit of 30050.3 birr ha^{-1} was obtained from the control check.

The maximum seed yield of chickpea and net benefit was obtained at the 30 kg S ha^{-1}when integrated with *Rhizobium* inoculation. Tentatively, combined application of 30 kg S with *Rhizobium* inoculation of the seed of chickpea is recommended for Gonder Zuria Woreda. However, it is difficult to make a definite and reliable conclusion based on two locations and one season experiment.

Attention shall be given to the following issue for future research:

- Conducting similar research over locations and seasons would be relevant to get conclusive result before recommending *Rhizobium* inoculation in the study site.
- The effectiveness native rhizobia nodulating chickpea should be evaluated over location and the relationship with native rhizobia, soil fertility status and cropping system need further investigation.
- Other better effective rhizobia based inoculant should be developed and tested

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7. APPENDICES

Appendix Table 1. Mean square values of nodule number, nodule volume and nodule dry weight per plant.

Source of Variation	df	NNPP (no plant^{-1})			NVPP (cc plant^{-1})			NDWPP (mg plant^{-1})		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	5.527	4.618	4.793	0.005	0.022	0.010	2.19	4.69	0.549
Inoculation	1	13.110**	7.747**	0.175^{ns}	0.019*	0.134*	0.064**	75.11**	289.00**	17.361*
S	2	1.013^{ns}	5.819**	2.614*	0.085**	0.299*	0.175**	25.86**	127.44**	33.465**
Zn	1	1.925^{ns}	32.680**	12.617**	0.020*	0.090*	0.049*	93.44**	93.44**	0.000^{ns}
Inoculation x S	2	2.866*	9.269**	3.193*	0.020*	0.060^{ns}	0.037*	312.53**	72.33**	124.465**
Inoculation x Zn	1	6.481*	0.100^{ns}	1.242^{ns}	0.018*	0.054^{ns}	0.034*	152.11**	25.00^{ns}	75.111**
S x Zn	2	19.348**	6.325**	11.870**	0.181**	0.528**	0.332**	564.19**	377.44**	463.521**
Inoculation x S x Zn	2	6.530*	4.795**	5.613**	0.101**	0.284**	0.181**	91.19**	394.33**	204.215**
Error	22	0.898	0.889	0.602	0.004	0.020	0.008	6.68	7.27	3.208
Total	35									

df=degree of freedom, NNPP =nodule number per plant (no plant^{-1}), NVPP=nodule volume per plant (cc plant^{-1}), NDWPP=nodule dry weight per plant (mg plant^{-1}), **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 2. Mean square values of effectiveness of nodules, nodulation rating and plant height.

Source of Variation	df	EN			NR			PH (cm)		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	0.019	0.411	0.128	0.112	0.025	0.059	1.7	1.2	0.013
Inoculation	1	0.034^{ns}	0.004^{ns}	0.015^{ns}	25.167**	41.818**	32.967^{ns}	3.4*	2.9*	0.010^{ns}
S	2	0.025^{ns}	0.005^{ns}	0.011^{ns}	3.394**	2.968**	3.154^{ns}	40.4**	13.5*	7.247**
Zn	1	0.001^{ns}	0.119^{ns}	0.026^{ns}	0.007^{ns}	0.040^{ns}	0.020^{ns}	6.5**	0.2^{ns}	1.027*
Inoculation x S	2	0.003^{ns}	0.064^{ns}	0.014^{ns}	0.045^{ns}	2.341**	0.759*	4.9**	8.3**	5.915**
Inoculation x Zn	1	0.020^{ns}	0.032^{ns}	0.026^{ns}	3.300**	0.871**	1.891*	1.2^{ns}	0.8^{ns}	0.004^{ns}
S x Zn	2	0.016^{ns}	0.023^{ns}	0.019^{ns}	3.875**	0.090^{ns}	1.128*	0.1^{ns}	14.7**	3.917**
Inoculation x S x Zn	2	0.068*	0.187*	0.117*	1.070**	3.334**	1.858*	0.7^{ns}	0.8^{ns}	0.512^{ns}
Error	22	0.013	0.037	0.018	0.068	0.027	0.015	0.4	0.4	0.203
Total	35									

df=degree of freedom, EN =effectiveness of nodule, NR=nodulation rating, PH=plant height (cm), **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 3. Mean square values of root length, shoot dry weight and root dry weight

Source of Variation	df	RL (cm)			SDW (gm plant^{-1})			RDW (g plant^{-1})		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	1.54	4.38	0.316	0.082	0.21	0.017	0.003	0.003	0.001
Inoculation	1	6.22^{*}	11.22^{**}	8.791^{**}	0.254^{ns}	0.75^{ns}	0.476^{ns}	0.006^{*}	0.002^{ns}	0.004^{**}
S	2	9.53^{**}	1.87^{*}	2.945^{**}	0.641^{ns}	9.08^{**}	3.299^{**}	0.005^{*}	0.014^{**}	0.001^{ns}
Zn	1	0.11^{ns}	0.67^{ns}	0.083^{ns}	1.638^{ns}	0.03^{ns}	0.531^{ns}	0.003^{ns}	0.006^{*}	0.004^{**}
Inoculation x S	2	1.41^{ns}	5.32^{**}	2.779^{**}	0.808^{ns}	1.58^{**}	0.843^{*}	0^{ns}	0.017^{**}	0.005^{**}
Inoculation x Zn	1	6.56^{*}	0.27^{ns}	2.223^{*}	0.220^{ns}	2.35^{**}	0.985^{*}	0.05^{**}	0.006^{*}	0.022^{**}
S x Zn	2	2.64^{*}	4.60^{**}	0.097^{ns}	1.188^{ns}	2.73^{**}	1.857^{**}	0.022^{**}	0.035^{**}	0.013^{**}
Inoculation x S x Zn	2	4.1^{**}	3.30^{*}	2.945^{**}	0.940^{ns}	2.39^{**}	0.835^{*}	0.043^{**}	0.004^{*}	0.010^{**}
Error	22	0.79	0.55	0.391	0.487	0.2	0.227	0.001	0.001	0
Total	35									

df=degree of freedom, RL =root length (cm), SDW=shoot dry weight (g plant^{-1}), RDW=root dry weight (g plant^{-1}), **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 4. Mean square values of number of primary branches and number of pod per plant

Source of Variation	df	NPB (no plant-1)			NPPP (no plant^{-1})		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	0.299	0.013	0.1	6.09	3.5	3.992
Inoculation	1	0.002^{ns}	0.040^{ns}	0.007^{ns}	3.63^{ns}	3.5^{ns}	3.511^{ns}
S	2	0.543^{**}	0.779^{**}	0.654^{**}	31.04^{**}	128.9^{**}	71.361^{**}
Zn	1	0.496^{*}	0.054^{ns}	0.055^{ns}	34.67^{**}	47.2^{**}	0.241^{ns}
Inoculation x S	2	0.309^{*}	0.276^{**}	0.013^{ns}	58.47^{**}	8.4^{ns}	21.013^{**}
Inoculation x Zn	1	0.457^{*}	0.160^{*}	0.291^{**}	2.61^{ns}	34.4^{**}	4.529^{ns}
S x Zn	2	0.125^{ns}	0.384^{**}	0.035^{ns}	0.05^{ns}	79^{**}	18.857^{**}
Inoculation x S x Zn	2	0.316^{*}	0.801^{**}	0.335^{**}	4.12^{*}	53.1^{**}	20.290^{**}
Error	22	0.065	0.036	0.022	1.13	3.4	1.356
Total	35						

df=degree of freedom, NPB =number of primary branch (no plant^{-1}), NPPP=number of pod per plant (no plant^{-1}), **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 5. Mean square values of number of seed, hundred seed weight and days to flowering.

Source of Variation	df	NSPP (no pod^{-1})			HSW (gm 100 seed)			DF		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	0.007	0.007	0.002	0.4	0.12	0.119	0.58	0.27	1.694
Inoculation	1	0.012^{ns}	0.003^{ns}	0.001^{ns}	1.6**	0.02^{ns}	0.467*	3.361^{ns}	5.44*	0.007^{ns}
S	2	0.005^{ns}	0.060**	0.010^{ns}	0.38*	1.15*	0.074^{ns}	5.778*	0.08^{ns}	1.361^{ns}
Zn	1	0.003^{ns}	0.003^{ns}	0.003^{ns}	1.43**	0.61^{ns}	1.007**	1.361^{ns}	2.78*	1.174^{ns}
Inoculation x S	2	0.003^{ns}	0.003^{ns}	0.002^{ns}	2.27**	0.24^{ns}	0.807**	10.111**	0.19^{ns}	3.444^{ns}
Inoculation x Zn	1	0.001^{ns}	0.007^{ns}	0.001^{ns}	1.85**	0.99*	1.418**	0.028^{ns}	18.78**	5.840^{ns}
S x Zn	2	0.023*	0.023*	0.009^{ns}	0.54*	0.77*	0.488**	0.111^{ns}	0.36^{ns}	4.528^{ns}
Inoculation x S x Zn	2	0.001^{ns}	0.037**	0.022**	0.31*	1.90**	0.529**	8.778**	15.36**	0.778^{ns}
Error	22	0.005	0.004	0.002	0.08	0.18	0.051	0.891	0.59	2.149
Total	35									

df=degree of freedom, NSPP =number of seed per pod (no plant^{-1}), HSW=hundred seed weight (g 100 seed), DF=dates of 50% flowering, **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 6. Mean square values of seeds yield, straw yield and harvest index

Source of Variation	df	GY (kg ha^{-1})			SY (kg ha^{-1})			HI		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	6465.7	9459	1763	3303.3	2979	2532	0.00064	0.00121	0.000112
Inoculation	1	6139.7^{ns}	16826^{ns}	455^{ns}	19111.9*	5786^{ns}	11393^{ns}	0.00112*	0.000086^{ns}	0.000153^{ns}
S	2	87622.6**	200344**	135520**	10816.5^{ns}	68025**	32119^{ns}	0.000349^{ns}	0.000982^{ns}	0.000045^{ns}
Zn	1	18.2^{ns}	13298^{ns}	2621^{ns}	61639.2**	55005*	56^{ns}	0.00355**	0.00052^{ns}	0.00021^{ns}
Inoculation x S	2	19273.8^{ns}	15351^{ns}	5146^{ns}	59306**	42681*	48801**	0.00105*	0.00105^{ns}	0.000917*
Inoculation x Zn	1	9283^{ns}	11431^{ns}	87^{ns}	1.3^{ns}	6389^{ns}	1662^{ns}	0.00053^{ns}	0.0000121^{ns}	0.000046^{ns}
S x Zn	2	45861.8*	42493*	40436*	15665.2*	171400**	39889**	0.00106*	0.00202^{ns}	0.000042^{ns}
Inoculation x S x Zn	2	39886.9*	52565*	34978*	4280.6^{ns}	61494**	11741*	0.00078*	0.00681*	0.00132**
Error	22	11908.7	12131	6116	3817.5	2979	2839	0.00021	0.00173	0.000142
Total	35									

df=degree of freedom, GY =seed yield (kg ha-1), SY=straw yield (kg ha^{-1}), HI= Harvest Index, **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 7. Mean square values of total nitrogen and phosphorus uptake

Source of Variation	df	TNU (kg ha^{-1})			TPU (kg ha^{-1})		
		Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Replication	2	15.9	1.4	6.7	0.16	0.17	0.02
Inoculation	1	3.7^{ns}	54.3**	21.7*	6.27**	0.06^{ns}	1.266**
S	2	310.9**	569.2**	429.5**	42.28**	18.52**	29.193**
Zn	1	24.3^{ns}	77.8**	47.3**	2.52**	4.85**	3.577**
Inoculation x S	2	26.3^{ns}	3.3^{ns}	9.9*	0.97**	2.06**	1.395**
Inoculation x Zn	1	4.6^{ns}	10.4*	7.1^{ns}	0.93*	0.02^{ns}	0.302^{ns}
S x Zn	2	42.9*	46.9**	10.0*	2.05**	11.65**	3.019**
Inoculation x S x Zn	2	29.8**	0.03*	7.1*	0.55*	3.04**	0.344*
Error	22	8.2	2	2.3	0.1	0.3	0.09
Total	35						

df=degree of freedom, TNU =total nitrogen uptake (kg ha^{-1}), TPU=total phosphorus uptake (kg ha^{-1}), **, * and NS = significant at 1%, 5% and non-significant, respectively.

Appendix Table 8. Correlation analysis of soil before planting

	pH	CEC	EC	Na	K	Ca	Mg	OM	N	P	S	Fe	Cu	Mn	Zn
pH	1														
CEC	0.58	1													
EC	0.84	0.25	1												
		0.69													
Na	0.77	0.22	0.92*	1											
		0.73	0.03												
K	-0.25	-0.37	-0.43	-0.12	1										
		0.54	0.47	0.84											
Ca	0.89*	0.81	0.77	0.71	-0.49	1									
		0.10	0.13	0.18	0.41										
Mg	-0.75	0.07	-0.86	-0.88*	-0.05	-0.49	1								
		0.92	0.06	0.05	0.94	0.40									
OM	0.43	-0.45	0.66	0.52	-0.11	0.11	-0.81	1							
		0.45	0.22	0.37	0.86	0.86	0.10								
N	0.84	0.25	1.00**	.919*	-0.43	0.77	-0.86	0.66	1						
		0.69	0	0.03	0.47	0.13	0.06	0.22							
P	-0.48	-0.39	-0.67	-0.40	0.96*	-0.65	0.23	-0.30	-0.67	1					
		0.52	0.21	0.50	0.01	0.23	0.70	0.63	0.21						
S	0.32	0.02	0.65	0.83	-0.11	0.42	-0.57	0.23	0.65	-0.31	1				
		0.97	0.23	0.09	0.86	0.49	0.32	0.71	0.23	0.61					
Fe	-0.80	-0.72	-0.71	-0.48	0.75	-0.89*	0.35	-0.21	-0.71	0.84	-0.15	1			
		0.17	0.18	0.42	0.14	0.04	0.57	0.74	0.18	0.08	0.82				
Cu	-0.54	-0.01	-0.81	-.952*	0.04	-0.51	0.81	-0.48	-0.81	0.30	-0.94*	0.25	1		
		0.99	0.10	0.01	0.95	0.39	0.10	0.42	0.10	0.62	0.02	0.69			
Mn	-0.77	-0.27	-0.57	-0.73	-0.40	-0.54	0.79	-0.36	-0.57	-0.15	-0.36	0.25	0.59	1	
		0.66	0.32	0.16	0.50	0.35	0.11	0.56	0.32	0.82	0.56	0.68	0.29		
Zn	0.00	0.44	-0.32	0	0.58	0.11	0.21	-0.70	-0.32	0.57	0.08	0.25	0	-.379	1
		0.46	0.60	1	0.30	0.86	0.74	0.19	0.60	0.31	0.90	0.69	1	.529	

**Significant at the 1% level; *Significant at the 5% level