

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

Beza Shewangizaw (✉ bezashewangezaw@gmail.com)

Amhara Agricultural Research Institute, Debra Birhan agricultural research Center

Anteneh Argaw

School of Natural Resource Management and Environmental science, Haramaya University

Tesfaye Feyisa

Amhara Agricultural Research Institute

Endalkachew Wold-Meskel

World Agroforestry (ICRAF)

Birhan Abdulkadir

International livestock Research Institute

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Abstract

In sub-Saharan Africa, multiple plant nutrients deficiency besides N and P is a major growth-limiting factor for crop production. As a result, some soils become non-responsive for *Rhizobium* inoculation besides P application. Based on the soil test result, the soil of experimental sites had low OM, N, P, S and Zn. Hence, an 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, nodulation, N and P uptake of chickpea. 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. Results showed that the highest mean nodule number (15.3) and nodule volume (1.3 ml plant⁻¹) over locations were obtained with *Rhizobium* inoculation integrated with 15 kg S and 1.5 kg Zn ha⁻¹ which resulted in 37.8% and 116.7% increment over the control check, respectively. It was also observed that combined application of *Rhizobium* and 30 kg S ha⁻¹ caused the highest (6.7) mean nodulation rating and seed yield (1775.5 kg ha⁻¹) over locations which resulted in 86.1% and 28 % increase over the control check, respectively. Moreover, this treatment improved P use efficiency of chickpea. On the bases of observed result, it can be concluded that the response of chickpea to *Rhizobium* and P application can be improved by S application and *Rhizobium* inoculation with application of 30 kg S ha⁻¹ with recommended rate of P and starter N is recommended for chickpea production at the experimental locations in Gonder Zuria Woreda.

Introduction

Nitrogen deficiency is a major factor limiting crop production in the tropics and subtropics (Wolde-meskel, 2007). To alleviate this limitation, the use of inorganic fertilizers by African farmers is limited as a result of high prices which is most of the time unaffordable by the subsistent farmers (Bagayoko et al., 2011). As a result, searching environmentally friendly and economically sound strategy is undeniably important (Lee and Song, 2007; Rigby and Cáceres, 2001). In this region, leguminous crops are extensively cultivated for human consumption. Moreover, this crops have the ability to reduce atmospheric N₂ to usable form when it forms an association with root nodule inducing bacteria (Adjei et al., 2001). The occurrence of effective rhizobia in the soil is prerequisite for efficient legume-rhizobia symbiosis to deliver high N to the host plant and to enrich the soil N for the preceded crops (Choudhry, 2012). When the soil harbour ineffective in N₂ fixation as well as insufficient number of rhizobia below 100 rhizobia g⁻¹ of soil, exogenous application of effective rhizobia is essential (Singleton and Tavares, 1986).

Chickpea (*Cicer arietinum* L.) is the second important pulse crops that belongs to the legume family. The crop is mainly produced for human consumption, animal feed and as a rotational crop with cereal. Chickpea is one of the health food that provide cheap but high quality protein especially for those developing countries that can't afford high price for animal protein. Moreover, it is a good source of carbohydrates, minerals and trace elements. On average dry chickpea kernels contains 56% fat, 47% starch, 23% protein, 6% soluble sugar, 6% crude fiber and 3% ash (Goa, 2014). Similarly, chickpea is an

important crop in Ethiopia. In this country, chickpea productivity is the highest compared with the top ten producing countries of the world with contribution of about 2% of the total world chickpea production. It is also the largest producer of the crop in Africa accounting for about 46% of the production (Kassie et al., 2009). In Ethiopia, this crop shares 14% of the total area and 15% of the total production from major pulse crops.

For the last thirty years, several promising results have been obtained with inoculation under greenhouse as well as field condition in different plant species in Ethiopia (Beyene, 1988). Likewise, studies showed a positive influence of rhizobia inoculation in combination with NP fertilizer on chickpea. However, the productivity of chickpea after inoculation when compared with the productivity reported elsewhere as potential yield is very low. For example, the national average productivity of chickpea (1.89 tone ha) (CSA, 2014) was still lower than its potential yield (5.5 tone ha) (Belay, 2006) obtained on experimental stations. Such wide yield gap clearly indicates that research on chickpea should be focused on the development of appropriate technology for other constraints such as limited plant nutrient on top of developing improved varieties for higher yield and disease resistance.

An adequate supply of mineral nutrients to legumes enhances nitrogen (N) fixation and yield (Ganettmfshamurthy and Sammi Reddy, 2000). In the previous experiment in Gondar Zuria Woreda (Dinzaz, Degolla and Tsion Kebeles) revealed that there was no significant effect of P fertilizer application and *Rhizobium* inoculation on chickpea. Based on the soil test result we did, the soil of the study sites are deficient in S and Zn in addition to N and P nutrients. But these nutrients are essential for both plant growth as well as the symbiosis between rhizobia and the host plant. Sulphur affects leguminous plant species growth since sulfur is important for nitrogenase (Mortenson and Thorneley, 1979) and ferredoxin (Yoch, 1979). Ferredoxin has a significant role in nitrogen dioxide and sulphate reduction, during assimilation of N by root nodule bacteria and free living N-fixing soil bacteria (Scherer et al., 2008). Zinc is also involved in various host plant metabolic processes, nodule growth and N₂ fixation process. Zn has also important role in activating plant enzymatic system, synthesis of chlorophyll and carbohydrates. Therefore, mitigating the S and Zn deficient in the soils of the study sites, we hypothesized that one way of improving the effective rhizobia in combination with P thereby enhancing the productivity of chickpea. 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.

Materials And Methods

Study sites

This on farm experiment was conducted in Gondar Zuria District, Tsion Siguaje and Denzaz Kebele.

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, mheat and, barley (GOZOARD, 2016). The dominant soil type covering 80 % is Vertisols followed by 15% Nitisols and 5% Cambisols. Specifically, this on farm experiment was conducted at 37°33'33.9"E-37°33'34.1"E longitude and 12°25'00.9"N-12°25'00.93"N latitude with an elevation of 1924m.

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. Specifically, this on farm experiment was conducted at 37°36'24.9"E-37°36'25.01"E longitude and 12°25'08.1"N-12°25'08.13"N latitude with an elevation of 2037m.

The growth period extends from September to January. The total amount of rainfall during the growth period was 142.6 mm, which is sufficient for the crop growth (fig.1A). The mean maximum and mean minimum temperature recorded were 27.7 and 12.2°C, respectively. The maximum and minimum temperature recorded during the growth period were 30.1 and 7.6°C, respectively (fig. 2B).

Soil and Plant Sample Collection and Processing

To identify the possible yield limiting essential plant nutrient in the study sites and hence to set up the treatment, soil samples were collected from those experimental sites on which diagnosis and demonstration of P fertilizer and *Rhizobium* inoculation trial were implemented by N2 Africa project. Five separate composite surface soil samples (0-20 cm depth) were collected from Gondar Zuria woreda (Tsion, Denzaz and Degola 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 standard sieve. The samples were then analyzed for texture, pH, TN, OC, CEC, exchangeable cations (Ca, Mg, K, and Na) extractable P, extractable S, and micronutrients (Zn, Fe, Cu and Mn). The numbers of indigenous rhizobia nodulating chickpea (*Cicer artemium* L.) present in the soils of the study sites that could nodulate chickpea was estimated by the most-probable-number (MPN), plant infection technique (Somasegaran and Hoben, 1994).

At physiological maturity, five randomly selected plants were harvested at the ground level and partitioned in to grain and haulm (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 haulm.

Experimental set-up

The experiment comprised of three factors with two levels of *Rhizobium* inoculation (+I = *Rhizobium* inoculated and -I = Un-inoculated), three levels of sulphur (0, 15 and 30 kg S ha) and two levels of Zn (0 and 1.5 kg Zn ha). The factorial combinations of the three factors were laid in RCB design with three replications.

Treatment combinations

1. Control check
2. *Rhizobium* inoculation alone (+I)
3. 1.5 kg ha Zinc alone (-I*1.5Zn)
4. 15 kg ha Sulphur alone (-I*15S)
5. 30 kg ha Sulphur alone (-I*30S)
6. *Rhizobium* inoculation +15 kg ha Sulphur (+I*15S)
7. *Rhizobium* inoculation + 30 kg ha Sulphur (+I*30S)
8. *Rhizobium* inoculation + 1.5 kg ha Zinc (+I*1.5Zn)
9. 15 kg ha Sulphur +1.5 kg ha Zinc (-I*15S*1.5Zn)
10. 30 kg ha Sulphur + 1.5 kg ha Zinc (-I*30S*1.5Zn)
11. *Rhizobium* inoculation + 15 kg ha Sulphur + 1.5 kg ha Zinc (+I*15S*1.5Zn)
12. *Rhizobium* inoculation + 30 kg ha Sulphur + 1.5 kg ha Zinc (+I*30S*1.5Zn)

Moreover, negative treatment without inorganic fertilizer (including starter N and P) and *Rhizobium* inoculation was included as a satellite treatment for the determination of nutrient uptake and hence nutrient efficiency determination.

The experimental sites were prepared using standard cultivation practices before planting. Trial fields were plowed using oxen-drawn implements at the depth of about 10 cm for first plowing and 15 cm for the last pass. Sowing was done on September 13 and 15/2016 at Denzaz and Tsion, respectively. Improved chickpea varieties (Arerti) was selected based on the recommendation of research because of local adaptation and market preferences. This variety are relatively long duration (105-155 days) relative to other kabuli type chickpea released in Ethiopia. The plot size used was 3 m x 3.4 m (10.2 m²). 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 (6.12m²) was used for the final harvest. The spacing between each treatment and block were 1 m and 1.5 m, respectively. Ridge was constructed between plot and plot to remove cross contamination of fertilizer and inoculant between plot and ridge.

All treatments (except the negative control) received equal amount of starter inorganic 20 kg N ha⁻¹ (Argaw and Muleta, 2016), 20 kg P ha⁻¹ (Ahlawat and Ali, 1993; Meen et al., 2010) in the form of Urea and

Triple super phosphate, respectively. Sulfur and zinc fertilizer were applied in the form of calcium sulfate and zinc sulfate, respectively. Zinc sulfate was applied on foliar parts (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).

Chickpea Mesorhizobium strain CP-41 that was selected based on its ability to enhance nodulation and grain yield under wide ecological condition (Funga et al., 2016; Tena et al., 2016) was obtained from MBI (Menagesha Biotechnology Industry). 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 cross contamination, plots with un-inoculated seeds were planted first followed by the inoculated ones.

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. The sampled plants from each plot were used to record the following observations; nodule number, nodule dry weight and effectiveness of nodule. Other agronomic and yield related data were also collected on different growth stage as well as at harvest and after harvesting of the test crop.

At the end of the season, the central six rows from each plot (3.4 m × 1.8 m) was harvested at the ground level. Then, total biomass (the grain and haulm yield) was weighted at harvest. After threshing, seeds were cleaned and weighed. Seed moisture content was measured using a gravimetric method. Total biomass (on dry matter basis) and grain yields were adjusted to a moisture content of 12.5%. Haulm yield was obtained by subtracting grain yield from total biomass yield.

Phosphorus and Nitrogen uptake by seed and haulm was determined from the P and N content of respective part after multiplying the seed yield and haulm yield, respectively. Phosphorus Use Efficiency were calculated with the help of the following formula

$$\text{PUE} = \frac{\text{biological yield at higher P} - \text{biological yield at lower P}}{\text{P uptake in biomass at higher P} - \text{P uptake in biomass at lower P}} \quad (\text{Fageria and Santos, 2002})$$

Where PUE = Phosphorus use efficiency

Statistical analysis

The collected data were subjected to analyses of variance (ANOVA) to evaluate the treatment effect 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 (Gomez and Gomez, 1984).

Results And Discussion

Soil property

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. The soil pH of trial sites was ranged between 7.0 to 7.9 with a mean value of 7.4, which is neutral and ideal for the production of most field crops including chickpea. Based on the results obtained for soil analysis, the average total nitrogen (N), available phosphorus (P), available Sulfur (S) were found to be below the critical levels (Table 1); and not optimal for crop production. These values were 0.052%, 7.42 ppm and 7.5 ppm, respectively, for N, P and S. Thus, based on the rating developed by (Tekalign, 1991) for Ethiopian soil, the average total nitrogen content of the soil 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 (Hailu et al., 2015; Mengel and Kirkby, 1987; Mesfin, 1998). Similarly, based on the soil rating developed by Landon (1991), the available P was rated as low. Moreover, 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 it's potential to supply S to plant growth through mineralization is low. Other authors also reported deficiency in S in Vertisols of Ethiopia (EthioSIS, 2016; Fanuel, 2015; Habtamu et al., 2014; Hailu et al., 2015; Lelago et al., 2016).

The exchangeable cations ($EC=0.1$ dS/m) and cation exchange capacity ($CEC=58.7$ cmo (+) kg^{-1} soil) of the soil were also determined (Table 1). The average contents of potassium (K), calcium (Ca) and magnesium (Mg) in the soil were 0.8, 47.5 and 23 cmo(+) kg^{-1} soil, respectively (Table 1). Based on the rating developed for those nutrients it were found to be above the optimum range (Berhanu, 1985; Hazelton and Murphy, 2007). This result is also in agreement with former findings in different vertisols dominated areas of Ethiopia (Desta, 1982; Fassil and Charles, 2009; Hailu et al., 2015; Kamara et al., 1989; Lemma and Smit, 2008).

The average Fe, Cu and Mn of the soil were found above the critical range, which are 12.9, 2.4 and 18.7 $mg\ kg^{-1}$ soil respectively. In contrast, Zn deficiency is widespread in many of the world's major chickpea-growing areas (Cakmak et al., 1995). According to Ahlawat et al. (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). 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). Other research findings also confirmed deficiency of Zn in Vertisols of Ethiopia (EthioSIS, 2016; Haileselassie et al., 2011; Yifru and Mesfin, 2013).

Native rhizobia population

The MPN background rhizobia nodulating chickpea in Tsion and Denzaz site were varied between 17×10^{-1} to low ($<10 \times 10^{-1}$ rhizobia cells g^{-1} soil) though the districts have many years of experience in chickpea cultivation (IFPRI, 2015). Indicating that native rhizobia population is not abundant enough to initiate optimum nodulation and provide sufficient amount of N through BNF (Slattery et al., 2004).

Nodule number

Nodule number is one of the parameters in assessing the performance of nodules in accordance with their ability to fix atmospheric nitrogen. In this regard; application of *Rhizobium* inoculation, S and Zn was found to be significant on nodule number (Table 2). At Tsion site, the highest nodule number (15.7) was found to be significant on nodule number (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 obtained 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 ha^{-1} 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.

Table 1.

Physico-chemical properties of the soil before planting

Parameters	Before planting					Mean
	Sites					
	1/Degola ⁺	2/Degola ⁺	3/Tsion [*]	4/Denzaz ⁺	5/Denzaz [*]	
Textural class ^a	Clay	Clay	Clay	Clay	Clay	
pH (1:2.5 H ₂ O) ^b	7.3	7.0	7.9	7.6	7.0	7.4
Ec (dS/m) ^c	0.09	0.07	0.13	0.09	0.09	0.1
Organic Carbon (%) ^d	0.61	0.61	0.73	0.68	0.74	0.67
Total N (%) ^e	0.05	0.04	0.07	0.05	0.05	0.05
Available P (mg kg^{-1}) ^f	0.8	17.1	2.8	9.7	6.7	7.42
Available S (mg kg^{-1}) ^g	7.6	8.0	10.5	5.4	7.6	7.8
CEC(cmo (+) kg^{-1} soil) ^h	62.6	57.6	60.1	60.1	53.1	58.7
Na ⁺ (cmo(+) kg^{-1} soil) ⁱ	0.2	0.2	0.3	0.2	0.2	0.2
K ⁺ (cmo(+) kg^{-1} soil) ⁱ	0.6	1.1	0.8	0.9	0.8	0.8
Ca ²⁺ (cmo(+) kg^{-1} soil) ^g	51.7	41.4	56.7	48.6	39.2	47.5
Mg ²⁺ (cmo(+) kg^{-1} soil) ^g	27.1	25.4	16.9	22.6	23.1	23.0
Fe (mg kg^{-1} soil) ^k	8.4	20.5	8.3	10.8	16.7	12.9
Cu (mg kg^{-1} soil) ^k	2.7	2.5	1.5	2.9	2.5	2.4
Mn (mg kg^{-1} soil) ^k	24.5	18.1	11.3	15.5	24.2	18.7
Zn (mg kg^{-1} soil) ^k	0.5	0.6	0.5	0.5	0.4	0.5

Method: ^a Hydrometer; ^b Potentiometric-water extract; ^c conductivity-water extract; ^d Walkley and Black; ^e Kjeldahl; ^f Olsen; ^g Turbidimetric; ^h Ammonium acetate; ⁱ Ammonium Acetate Extract-Flame photometry; ^g Ammonium Acetate Extract- EDTA Titration; ^k Diehyleneetriaminepentaacetic acid (DTPA).

⁺ indicates farmers field on which soil samples collected for identification of limited plant nutrient, ^{*} indicates farmers field soil samples collected as well as on farm experiment conducted.

Nodule dry weight

In the case of nodule dry weight, the highest and lowest value at Tsion 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 (Table 2). At Denzaz, the highest (46.7 mg plant⁻¹) and lowest (14.7 mg plant⁻¹) nodule dry weight were obtained from *Rhizobium* inoculation when integrated with 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 which resulted in 22.2% increase over the control check.

Effectiveness of nodules

The effectiveness of nodules in its ability to fix atmospheric nitrogen in response to inoculation, S and Zn was assessed using nodule color. Nodule color was found to ranged 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 2.

Number of nodule, nodule dry weight and Effectiveness of nodule as affected by *Rhizobium*, S and Zn rates

Treatment	NNPP			NDWPP (mg plant ⁻¹)			EN		
	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Control check	13 ^{de}	10.5 ^{cd}	11.1 ^{de}	52 ^b	27.3 ^{bcd}	39.7 ^c	2.8 ^a	2.8 ^{ab}	2.8 ^a
-I*1.5Zn	15.6 ^{ab}	10.5 ^{bcd}	13.8 ^b	50.3 ^{bc}	14.7 ⁱ	32.5 ^d	2.7 ^{ab}	2.7 ^{abc}	2.7 ^{abcd}
-I*15S	12.9 ^{def}	11.1 ^{bc}	11.2 ^{cde}	47 ^{cd}	26.7 ^{cde}	36.8 ^c	2.5 ^{bc}	2.4 ^c	2.5 ^d
-I*15S*1.5Zn	15.7 ^a	11.9 ^{efg}	13.7 ^b	37.3 ^e	19.3 ^{gh}	28.3 ^f	2.7 ^{ab}	2.8 ^{ab}	2.8 ^{abc}
-I*30S	14 ^{bcd}	10.7 ^{bcd}	12.5 ^{bc}	39 ^e	22.7 ^{efg}	30.8 ^{def}	2.6 ^{ab}	2.6 ^{abc}	2.6 ^{abcd}
-I*30S*1.5Zn	12.4 ^{defg}	11.9 ^{bc}	11.9 ^{cde}	47.7 ^{bcd}	28 ^{bcd}	37.8 ^c	2.7 ^{ab}	2.9 ^a	2.8 ^a
+I	13.4 ^{cde}	9.3 ^d	11.9 ^{cde}	29.3 ^f	16 ^{hi}	22.7 ^g	2.6 ^{ab}	2.6 ^{abc}	2.6 ^{abcd}
+I*1.5Zn	10.9 ^g	12.1 ^b	10.7 ^e	44 ^d	31.3 ^b	37.7 ^c	2.6 ^{ab}	2.8 ^{ab}	2.7 ^{abc}
+I*15S	11.3 ^{fg}	9.3 ^d	11.2 ^{cde}	50.3 ^{bc}	46.7 ^a	48.5 ^a	2.7 ^{ab}	2.9 ^{ab}	2.8 ^{ab}
+I*15S*1.5Zn	14.7 ^{abc}	11.7 ^{bc}	15.3 ^a	36.3 ^e	22 ^{eg}	29.2 ^{ef}	2.4 ^c	2.7 ^{abc}	2.5 ^{cd}
+I*30S	14 ^{bcd}	11.1 ^{bc}	12.3 ^{cd}	37.3 ^e	26 ^{def}	31.7 ^{de}	2.6 ^{abc}	2.5 ^{bc}	2.6 ^{bcd}
+I*30S*1.5Zn	11.9 ^{efg}	11.4 ^{bc}	11.9 ^{cde}	58.7 ^a	30.7 ^{bc}	44.7 ^b	2.7 ^{ab}	2.7 ^{abc}	2.7 ^{abcd}
P-value	*	**	**	**	**	**	*	*	*
CV(%)	7.12	8.34	6.31	5.86	10.39	5.11	4.41	7.12	5.1

a, b, c Mean value with different letters of superscript with in the column are significantly different (P<0.05), +I=*Rhizobium* inoculated, -I=un-inoculated, 15S= 15 kg S ha⁻¹, 30S= 30 kg S ha⁻¹, 0Zn= 0 kg Zn ha⁻¹, 1.5Zn=1.5 kg Zn ha⁻¹, **=significant at 1%, *=, 5%, ns=non-significant, CV = Coefficient of variation, NNPP=nodule number per plant, NDWPP=nodule dry weight per plant, EN=effectiveness of nodule.

Plant height, Number of primary branch and number of pod

The plant height which was obtained from both locations and its mean values combined over location was not significantly affected by the treatment (Table 3). Incontrast, number of primary branch and pod per plant were significantly affected by the treatment. 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 3). 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 (Das et al., 2016; Jadeja et al., 2016; Kesare, 2014; Namvar et al., 2011; Ram and Katiyar, 2013; Sharma and Room, 1997; Togay et al., 2008) have been reported.

Similarly, 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 (Muhammad 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-kade and Mona (2013) reported that combined application of S and Zn increase pods number. Other researchers (Das et al., 2016; Hussain et al., 2011; Jadeja et al., 2016; Kanase et al., 2006; Kayan et al., 2015; Kesare, 2014; Namvar et al., 2011; Nasreen and Farid, 2006; Nasri et al., 2011; S. Ram and Katiyar, 2013; Togay et al., 2008; Y Zhao et al., 2008) also reported that number of pod increased with *Rhizobium* inoculation, S and Zn application.

Table 3.

Plant height, number of primary branch and number of pod of chickpea as affected by *Rhizobium*, S and Zn rates

Treatment	PH (cm)			NPBPP			NPPP		
	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Control check	39.2	34.5	36.9	3.8 ^{cde}	2.1 ^g	2.9 ^f	48 ^{cd}	32.1 ^{ef}	40.1 ^{def}
-I*1.5Zn	39.3	32.3	35.7	3.4 ^e	3 ^{bcd}	3.4 ^{bc}	48.4 ^c	34.6 ^{cde}	41.5 ^{cde}
-I*15S	39.2	30.7	34.9	3.9 ^{bcd}	3.4 ^a	3.4 ^{bc}	44.4 ^{fg}	35.4 ^{cd}	39.9 ^{ef}
-I*15S*1.5Zn	39.8	30.9	35.4	3.7 ^{de}	2.6 ^{ef}	3.3 ^{bcd}	45.6 ^{ef}	33.1 ^{def}	39.3 ^f
-I*30S	39.5	32.7	36.1	4.5 ^a	3 ^{bc}	3.3 ^{bcd}	48.2 ^{cd}	38.6 ^b	43.4 ^{bc}
-I*30S*1.5Zn	40.4	35.1	37.8	3.5 ^e	3.1 ^b	3.7 ^a	51 ^a	37.5 ^{bc}	44.2 ^b
+I	36.4	33.9	35.2	3.5 ^e	2.7 ^{cdef}	3 ^{ef}	42.9 ^g	31.4 ^f	37.2 ^g
+I*1.5Zn	37.9	30.9	34.4	3.8 ^{bcd}	2.5 ^f	3.1 ^{def}	46.5 ^{de}	33.3 ^{def}	39.9 ^{ef}
+I*15S	39	32.7	35.9	4.3 ^{abc}	2.5 ^f	3.2 ^{cde}	49.1 ^{bc}	34.9 ^{cde}	42 ^{cd}
+I*15S*1.5Zn	40.6	33.5	37	4.2 ^{abc}	2.7 ^{def}	3.5 ^b	51.3 ^a	34.8 ^{cde}	43.1 ^{bc}
+I*30S	39.6	33.8	36.7	3.8 ^{bcd}	3.4 ^a	3.8 ^a	40 ^{bc}	47.7 ^a	48.3 ^a
+I*30S*1.5Zn	40.2	34.7	37.5	3.7 ^{de}	2.9 ^{bcd}	3.4 ^{bc}	50.4 ^{ab}	33 ^{def}	41.7 ^{bc}
LSD _{0.05}	ns	ns	ns	*	**	**	*	**	**
CV(%)	1.61	1.82	1.25	6.62	6.7	4.4	2.22	5.19	2.79

a, b, c Mean value with different letters of superscript with in the column are significantly different (P<0.05), +I=*Rhizobium* inoculated, -I=un-inoculated, 15S= 15 kg S ha⁻¹, 30S= 30 kg S ha⁻¹, 0Zn= 0 kg Zn ha⁻¹, 1.5Zn=1.5 kg Zn ha⁻¹, **=significant at 1%, *=, 5%, ns=non-significant, CV = Coefficient of variation, PH=plant height, NPBPP=number of primary branch per plant, NPPP= number of pod per plant

Grain yield

Increased chickpea grain yield due to soil fertility treatment was observed at both location and mean value combined over location (Table 3). At both sites, the highest (1515.2 at Dnzaz and 2039.8 kg ha at Tsion site) seed yield was obtained from *Rhizobium* inoculation integrated with 30 kg S ha⁻¹ whereas the lowest at Denzaz site and mean value combined over site were 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 3). The increase in yield might be due to the fact that S performs many physiological functions in cysteine, methionine and chlorophyll synthesis. *Rhizobium* inoculation also provides adequate supply of N for plant and resulted in increased chlorophyll synthesis and hence photosynthetic products including grain yield. This result was in agreement with some previous finding reported on the importance of combined application of S and *Rhizobium* inoculation in increasing seed yield (Islam et al., 2011; Kesare, 2014; Namvar et al., 2011; Namvar and Sharifi, 2011; Rokhzadi and Toashih, 2011; Srinivasarao et al., 2008; Togay et al., 2008; Zafar et al., 2014; Y Zhao et al., 2008; Kihara et al., 2017).

Haulm yield

Chickpea residue, locally known as “Defeka”, is important as a feed resource for livestock during the dry months of the year when green fodder is unavailable (Wolde-meskel et al., 2018). At Tsion site, the highest (1398.4 kg ha⁻¹) haulm 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 haulm 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 haulm yield was obtained from combined application of *Rhizobium* inoculation and 1.5 kg Zn ha⁻¹ which resulted in 27.6% Haulm yield advantage over the control check. In consistent with the current finding, Das et al. (2012) reported that *Rhizobium* inoculation with 10 kg ZnSO₄ and 25 kg ZnSO₄ ha⁻¹ resulted in increased haulm 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 Haulm 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 haulm yield of summer green gram.

Table 4.

Grain and Haulm yield as affected by *Rhizobium*, S and Zn rates

Treatment	Grain yield (kg ha ⁻¹)			Haulm yield (kg ha ⁻¹)		
	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Control check	1756.5 ^{cde}	1020.5 ^f	1421.8 ^{fg}	1160.1 ^d	987.8 ^e	1074 ^f
-I*1.5Zn	1726.6 ^{de}	1265.6 ^{cde}	1568.6 ^{cde}	1051.2 ^e	1212.1 ^{cd}	1131.7 ^{ef}
-I*15S	1963.5 ^{ab}	1307.2 ^{bcde}	1539.9 ^{def}	1269 ^{bc}	1206.2 ^{cd}	1237.6 ^{cd}
-I*15S*1.5Zn	1936.2 ^{abc}	1307.2 ^e	1666.3 ^{abcd}	1228.2 ^{cd}	1247.7 ^{abc}	1238 ^{abc}
-I*30S	1963.5 ^{ab}	1405.2 ^{abcd}	1777.5 ^a	1334.4 ^{ab}	1390.3 ^{ab}	1362.4 ^a
-I*30S*1.5Zn	1919.9 ^{abc}	1482.4 ^{ab}	1552.3 ^{cdef}	1236.4 ^{bcd}	1339.9 ^{bcd}	1288.1 ^{abc}
+I	1693.2 ^e	1150.3 ^{ef}	1388.5 ^g	1398.4 ^a	1015.4 ^e	1206.9 ^{de}
+I*1.5Zn	1895.4 ^{abcd}	1241.8 ^{de}	1496.1 ^{efg}	1250 ^{bcd}	1491.2 ^a	1370.6 ^a
+I*15S	1775.6 ^{cde}	1304.2 ^{bcde}	1635.4 ^{abcd}	1190.1 ^{cd}	1369.6 ^{ab}	1279.8 ^{bcd}
+I*15S*1.5Zn	1903.6 ^{abcd}	1429 ^{abc}	1621.7 ^{abcd}	1239.1 ^{bcd}	1194.3 ^d	1216.7 ^{cde}
+I*30S	2039.8 ^a	1515.2 ^a	1684.4 ^{abc}	1334.4 ^{ab}	1375.5 ^{abc}	1355 ^a
+I*30S*1.5Zn	1797.4 ^{bcde}	1307.2 ^{bcde}	1701.2 ^{ab}	1171 ^{cd}	1209.2 ^{cd}	1190.1 ^{de}
P-value	*	*	*	ns	**	*
CV(%)	5.85	8.45	4.94	5	7.14	4.27

a, b, c Mean value with different letters of superscript with in the column are significantly different ($P < 0.05$), +I=*Rhizobium* inoculated, -I=un-inoculated, 15S= 15 kg S ha⁻¹, 30S= 30 kg S ha⁻¹, 0Zn= 0 kg Zn ha⁻¹, 1.5Zn=1.5 kg Zn ha⁻¹, **=significant at 1%, *=, 5%, ns=non-significant, CV = Coefficient of variation.

Total nitrogen uptake

Total N uptake of legume can serve as a good indicator of N₂ fixation. Total N uptake was significantly improved with the fertility treatment (Table 4). The highest total N uptake (67.4 kg ha⁻¹) was generally observed in Tsion kebele. This was explained 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.

This increment could be attributed to *Rhizobium* inoculation helped in biological nitrogen fixation and thus, increase N content in grain and haulm. The increase in N uptake as a result of S application might be due to an increment in protein synthesis and enhance photosynthesis (Yanwen 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 (Abdalla et al., 2011; Das et al., 2016; Desta et al., 2015; El-kade and Mona, 2013; Hussain et al., 2011; Islam et al., 2013; Jay et al., 2012; Kesare, 2014; Mondal et al., 2005; Najjar et al., 2011; Rokhzadi and Toashih, 2011; Shamima and Farid, 2006; Srinivasulu et al., 2015; Srivastava et al., 2006; Togay et al., 2008; Zaidi et al., 2003; Zerihun et al., 2017)

Total phosphorus uptake

The analysis of variance showed that, application of S with inoculation and Zn were found to be significant (Table 4). At Tsion 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. At Denzaz, 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). The increase in P uptake in the present study agree with earlier findings reported by; Togay et al. (2008), Najjar et al. (2011), Yadav (2011), Islam et al. (2013), Kesare (2014), Das et al. (2016), Srinivasulu et al. (2015), Zerihun et al. (2017).

Table 5.

Total N and P uptake as affected by *Rhizobium*, S and Zn fertilizer rates

Treatment	TNU (kg ha ⁻¹)			TPU (kg ha ⁻¹)		
	Tsion	Denzaz	Mean	Tsion	Denzaz	Mean
Control check	49.8 ^g	30.8 ⁱ	40.3 ^g	8.4 ^f	8.4 ^f	8.4 ^f
-I*1.5Zn	52.3 ^{fg}	39.7 ^g	46 ^f	9.1 ^e	9.1 ^e	9.1 ^e
-I*15S	60 ^{bcd}	43.6 ^{ef}	51.8 ^e	12 ^b	12 ^b	12 ^b
-I*15S*1.5Zn	61.5 ^{bc}	44.1 ^{ef}	52.8 ^{de}	11.3 ^c	11.3 ^c	11.3 ^c
-I*30S	61.5 ^{bc}	48.6 ^{bc}	55 ^{cd}	12.7 ^b	12.7 ^b	12.7 ^b
-I*30S*1.5Zn	64.6 ^{ab}	51.3 ^a	58 ^{ab}	13.2 ^a	13.2 ^a	13.2 ^a
+I	56.6 ^{def}	35.6 ^h	46.1 ^f	8.2 ^f	8.2 ^f	8.2 ^f
+I*1.5Zn	53.4 ^{efg}	41.6 ^{fg}	47.5 ^f	8.6 ^{ef}	8.6 ^{ef}	8.6 ^{ef}
+I*15S	60.4 ^{bcd}	45.6 ^{de}	53 ^{cde}	10.8 ^{cd}	10.8 ^{cd}	10.8 ^{cd}
+I*15S*1.5Zn	57.3 ^{cde}	47.4 ^{cd}	52.3 ^{bde}	11 ^{cd}	11 ^{cd}	11 ^{cd}
+I*30S	58.4 ^{cd}	52.4 ^a	55.4 ^{bc}	10.7 ^d	10.7 ^d	10.7 ^d
+I*30S*1.5Zn	67.4 ^a	50.1 ^{ab}	58.7 ^a	12.5 ^b	12.5 ^b	12.5 ^b
P-value	**	*	*	*	**	*
CV(%)	4.88	3.21	2.95	3	3	3

a, b, c Mean value with different letters of superscript with in the column are significantly different (P<0.05), +I=*Rhizobium* inoculated, -I=un-inoculated, 15S= 15 kg S ha⁻¹, 30S= 30 kg S ha⁻¹, 0Zn= 0 kg Zn ha⁻¹, 1.5Zn=1.5 kg Zn ha⁻¹, **=significant at 1%, *=, 5%, ns=non-significant, CV = Coefficient of variation, TNU=total nitrogen uptake, TPU=total phosphorus uptake

P use efficiency

The result also demonstrated that at both locations, the highest P use efficiency (77.3% at Tsion and 163.6% at Denzaz site) were obtained with *Rhizobium* inoculation which resulted in 31% and 45.3% increase over the un-inoculated treatment (Fig. 4A). Application of 15 kg S ha⁻¹ also caused the highest P use efficiency and it increased P use efficiency by 12.6% over S control (Fig. 4B). 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. In contrast to this, Zn application did not affect P use efficiency (Fig. 4C). 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 et al., 2012).

Conclusion

In general, the use of effective rhizobia in combination with Zn and S in the both study sites increase the nodulation, yield and NP uptake and use efficacy of chickpea. Combined application of rhizobia and S could be the recommended for the integrated nutrient management for chickpea production in the study site.

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Figures

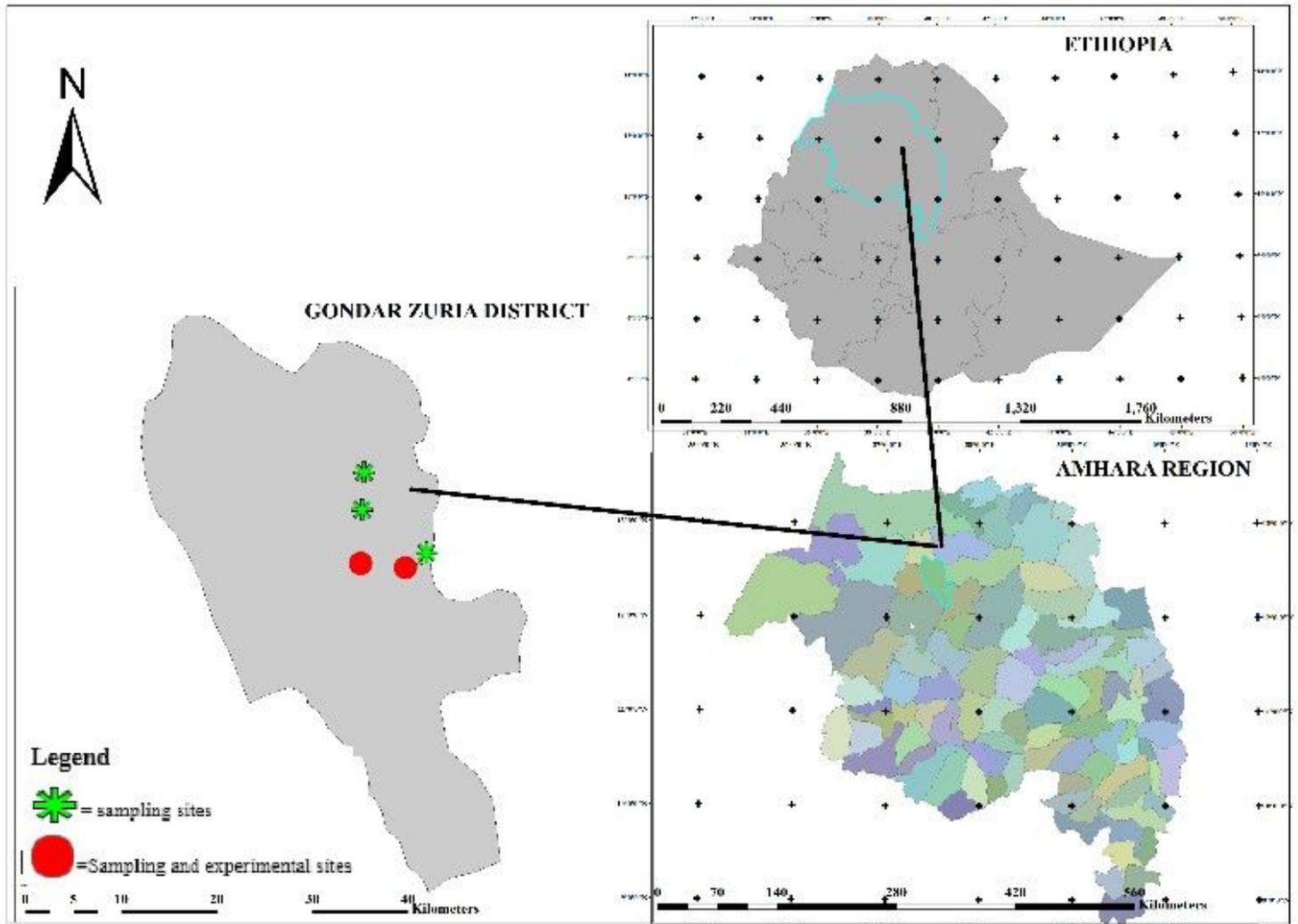


Figure 1

A map showing the location where soil sampling and the trial were conducted

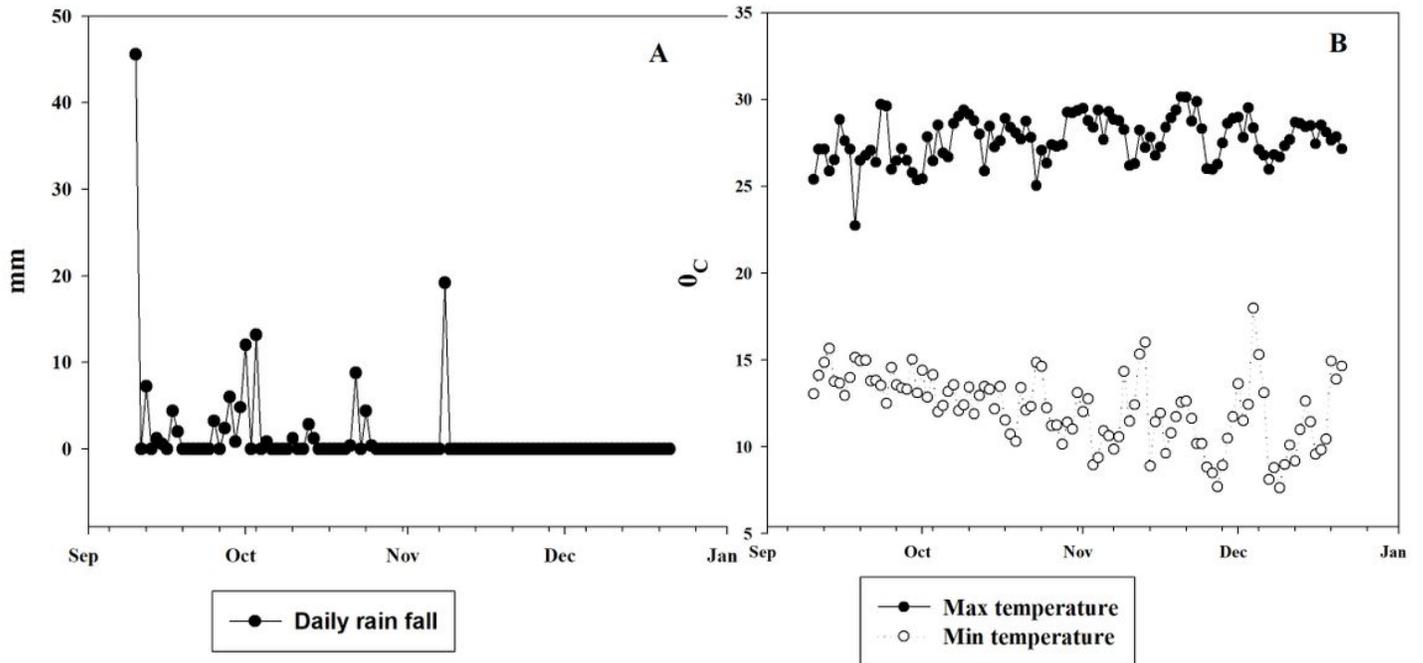


Figure 2

A, B, daily rain fall , maximum and minimum temperature during the growth period

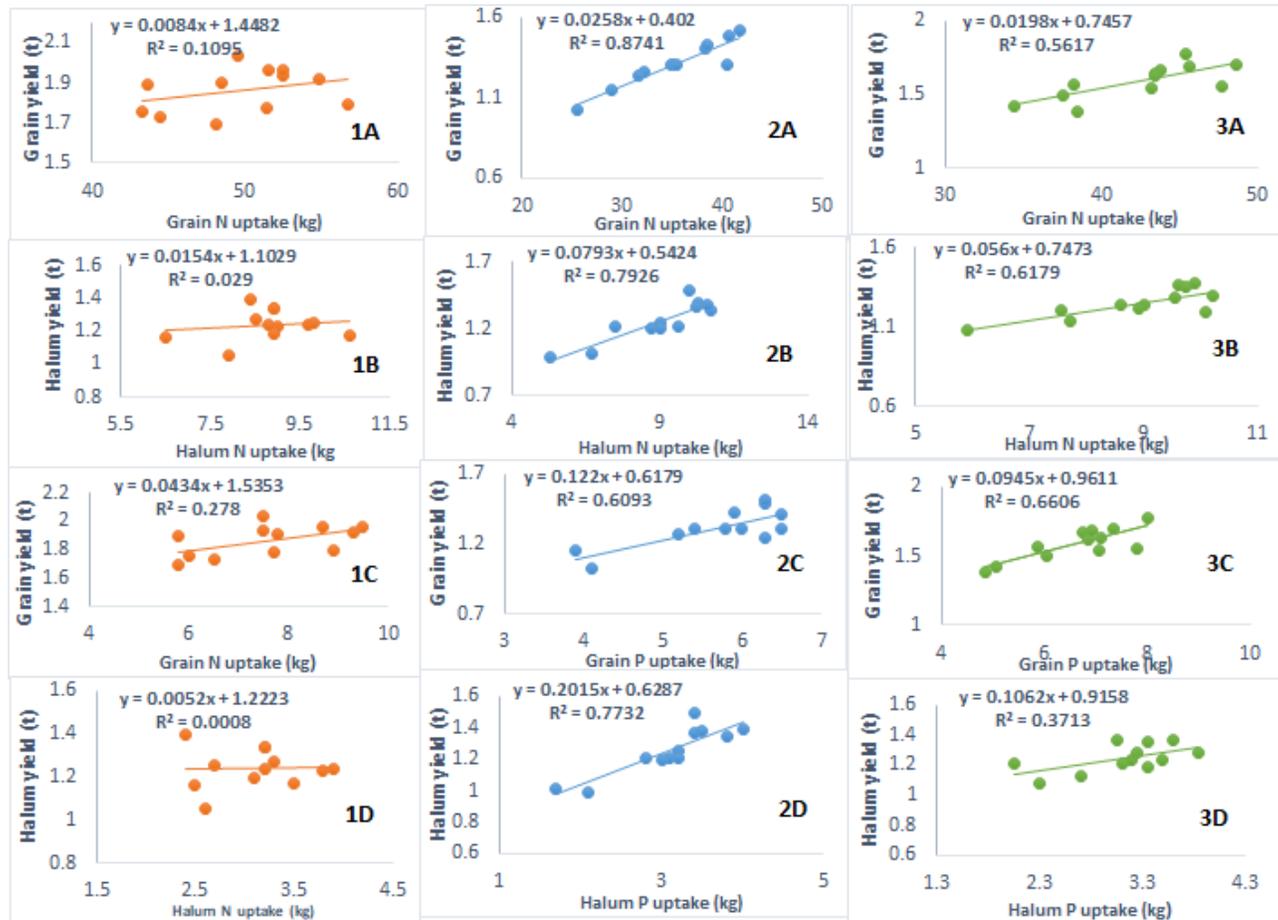


Figure 3

Correlation of biological yield with nutrient uptake (1,2,3=Tsion, denzaz and mean respectively; A,B,C,D= correlation of nutrient uptake with biological yield)

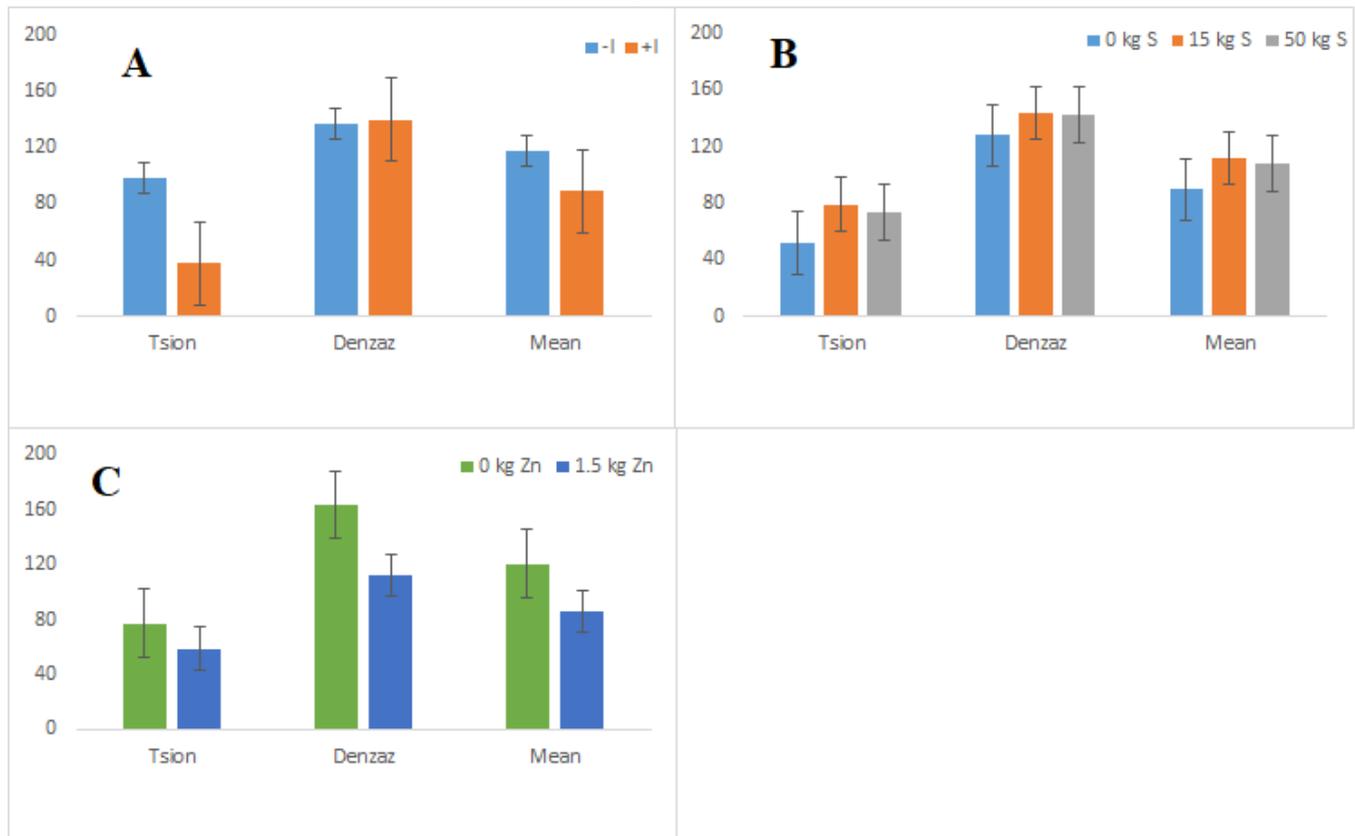


Figure 4

A, B and C- P use efficiency as influenced by level of Inoculation, S and Zn respectively