ORIGINAL ARTICLE

Effects of *mesorhizobium* inoculation, phosphorus and sulfur application on nodulation, growth and yield of chickpea (*Cicer arietinum* L.)

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ABSTRACT

Low soil fertility is one of the major constraints that limit crop production in sub-Saharan African countries, including Ethiopia. The field experiment was done during 2018/19 cropping season to assess the effects of Mesorhizobium inoculant, Phosphorus (P), and Sulfur (S) on nodulation, growth, nutrient uptake and yield response of chickpea. The experiment was laid out in a randomized complete block design (RCBD) with a factorial combination of Mesorhizobium inoculant (un-inoculated and inoculated), three levels of P (17, 34 and 51 kg/ha) and three levels of S (7, 14 and 21kg/ha) with three replications, and Haberu variety was used for all the experimental units. All plots received a basal application of 19 kg N/ha uniformly. The analysis of variance showed significant differences for the phenological, nodulation (nodule number and nodule dry weight), growth parameters (shoot dry weight, plant height and number of branches), yield and yield components and nutrient uptake of (N and P) traits in response to the main effects. The highest number (47) and dry weight (460 mg) of nodules per plant were found for the combined application of P (51 kg/ha) with Mesorhizobium inoculant. In addition, the application of 21 kg/ha S with Mesorhizobium inoculant gave 43 nodule number and 450.4 mg nodule dry weight and 13.1 gm shoot dry weight. The highest grain yield (2202.3 kg/ha), total N uptake (71.4 kg/ha), and total P uptake (15.4 kg/ha) were obtained from the application of 51 kg/ha P with Mesorhizobium inoculant. In addition, the highest total S uptake of 21.43 kg/ha was obtained from the application of 51 kg/ha P and 21 kg/ha S. Hence, it can be concluded that application 51 kg P/ha and 21 kg/ha S with inoculant is found to the best treatment combination. Thus, biofertilizer and appropriate fertilizer-P and S management will ensure optimal agronomic and economic returns in the study area.

Keywords: N and P uptake, Partial Budget Analysis, Rhizobia, Yield components

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop after beans and peas. These three pulses (beans, peas, and chickpeas) accounts for about 70% of the global pulse production with chickpea accounting for approximately 19% of the total annually (FAOSTAT, 2017). Chickpea is produced in over 50 countries with India having the largest production and accounting for over 61% of the total world production. Australia and Myanmar, the next most important producers, accounts for 13.5 and 3.5% of the global production, respectively. Ethiopia has emerged as a major and the first producing country in Africa (FAOSTAT, 2017).

Food legumes cover about 12.33% of the area under crop production in Ethiopia and contribute to nearly 9.69% of the total annual crops production (CSA, 2017). Chickpea production ranks second, next to common bean, among the pulses, with 225,607 ha of land under chickpea (15.55% of the area under pulse) with a total production of 444,145.9 ton and an average yield of 1.97 t/ha (CSA, 2017). On the other hand, the potential productivity of chickpea under improved management might reach 4-5 t/ha (Asnake, 2016).

Chickpea has an important role in the diet of smallscale farmers'. It serves as a source of income; its straw used for animal feed and improves the fertility status of soil through its biological nitrogen fixation (Wondwosen *et al.*, 2017). It is usually grown on Vertisols in Ethiopia, which might be considered highly productive soils, if its water logging and workability problems are managed properly. Many soils in the highlands of Ethiopia are inherently poor in available plant nutrients and organic matter (OM) content, and the application of nutrients are considered essential to improve crops production on these soils (Asmare *et al.*, 2015).

The major factors that are responsible for the low productivity of Chickpea in Ethiopia that limit achieving the potential yield includes: limited availability and weak adaption of high yielding improved cultivars, damage of pests and failure to follow the best agronomic management practices, nutrient imbalance and insufficient availability of effective indigenous or commercial *Mesorhizobium* strains of chickpea. Maintaining soil fertility and use of plant nutrient in balanced amount is one of the key components in increasing crop production and productivity. Hence, managing soil fertility is very crucial for improving chickpea crop productivity.

Most Ethiopian soils are poor in nitrogen (N), phosphorus (P), and sulfur (S) contents (Wassie and Tekalign, 2013). In addition, soils often cultivated with cereals are generally deficient in N-fixing bacteria (*Rhizobium* spp.), which contributes to the low yield of chickpea (Wondwosen *et al.*, 2016). Hence, it is necessary to evaluate the use of inorganic fertilizers

with rhizobial inoculant, which may be useful to enhance the productivity of chickpea.

As a legume crop, chickpea can fix up to 140 kg N/ha from air and meet most of its N requirement through symbiotic nitrogen fixation (Sheleme et al., 2015). It is selective in its symbiotic requirements, nodulating with only in association with effective and compatible Mesorhizobium strain (Wondwosen et al., 2016). The rest of N gained from soil inorganic N, mineralized organic matter, residual N from the previous and/or fertilizer application (Endalkachew et al., 2018). Sheleme et al. (2015) suggested that an adequate supply of mineral nutrients to legumes enhances N₂ fixation and yield. This is due to the role of these nutrients in both plant growth and the symbiosis between rhizobia bacteria and the host plant. Rhizobia inoculation, especially in combination with the application of S and P nutrient improved pulse crops productivity, compared to rhizobia inoculation alone (Bahure et al., 2016). Similarly, Singh et al. (2018) also showed that different doses of phosphorus, sulfur and seed inoculation with biofertilizers significantly influenced the grain yield of chickpea.

Mortena Jiru district located in Northern Shewa Zone of the Amhara National Regional State has a favorable environment for chickpea cultivation. Vertisols are the dominant soil type in the district. Most soils in Amhara National Regional State, including Mortena Jiru district are deficient in available S, N, and P (ATA, 2016). This might be due to extensive use of S free fertilizers (DAP and urea), smaller rate of S and P in NPS, intensive crop production, soil erosion, poor S, P, and N of the soils (Wassie and Tekalign, 2013). Hence, one of the major problems responsible for low chickpea productivity in the study area is depletion of soil fertility resulted from low organic matter content, intensive cultivation, and soil erosion. Despite the importance of the problem, there have not been research and development efforts to improve the productivity of chickpea crop through the use of Mesorhizobium inoculant with P and S fertilizers. Thus, this experiment was conducted to determine the effects of Mesorhizobium inoculation, S and P application on the growth, nodulation, yield and yield components of chickpea grown on famers' fields at Mortena-Jiru district.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted at Mortena Jiru district (Woyra Anba kebele), North Shewa Zone of the Amhara National Regional State of Ethiopia. Woyra Anba is located at 195 km northeast of Addis Ababa and its geographical position is 9° 52' N longitude and 39° 10' E latitude with an altitude of 2680 m.a.s.l. (Figure 1). The area is characterized by a unimodal rainfall pattern that receives mean annual rainfall of

966.11mm with mean monthly maximum and minimum temperatures of 21.06 and 9.47 °C, respectively (Figure 2). Vertisols, which are known for their high water-logging problem, are the dominant soil types in the district (ADSWE, 2011). The crops widely grown in the study area include wheat, teff, chickpea and lentil; whereas faba bean, grass pea and other crops have low area coverage.



Figure 1: The location map of the experimental site



Figure 2. The monthly mean rainfall (mm), maximum and minimum temperature (°C) of the study area for ten years (2009-2018). Source: National Meteorological Agency, Debre Berhan Agricultural Research Center

Soil Sample Collection, Preparation and Analysis

One composite surface soil sample was collected with an auger from a soil depth of 0-30 cm from 20 randomly selected points using the zigzag sampling pattern. For determination of selected physico-chemical properties, the soil was air-dried, ground, mixed thoroughly and passed through a 2 mm sieve for most parameters, except for organic carbon (OC) and total nitrogen (TN) that passed through 0.5 mm sieve. Soil bulk density was measured from undisturbed soil samples collected using a core sampler, which after drying the soil core samples to constant weight in an oven at 105°C as per the procedures described by Black (1965). Soil particle size distribution (texture) was analyzed by the hydrometer method (Day, 1965).

Soil pH was measured with digital pH meter potentiometerically in supernatant suspension of 1:2.5 soils to distilled water ratio (Van Reeuwijk, 1992). Organic carbon (OC) was determined using the wet oxidation method (Walkley and Black, 1934); where the carbon oxidized under standard conditions with potassium dichromate in sulfuric acid solution. Finally, the organic matter (OM) content of the soil was calculated by multiplying the percent OC by 1.724. Total N in the soil was measured using micro Kjeldhal method (Jackson, 1958). Available P was determined by the Olsen method using NaHCO3 as extracting solution (Olsen et al., 1954). Available sulfur was measured in the soil by mono-calcium phosphate extraction method (Hariram and Dwivedi, 1994). The exchangeable bases (Ca, Mg, Na and K) in the soil were determined from the leachate of 1 molar ammonium acetate (NH₄OAc) solution at pH 7.0. Exchangeable Ca and Mg were measured by atomic absorption spectrophotometer, while K and Na read using flame photometer (Rowell, 1994). The CEC was measured titrimetrically using distillation of ammonia that displaced by Na from NaCl solution (Chapman, 1965).

Treatments and experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times per treatment in a factorial combination. Three levels of P (P₁=17 kg/ha, P₂=34 kg/ha and P₃=51 kg/ha), three levels of S (S₁=7 kg/ha, S₂=14 kg/ha and $S_3=21$ kg/ha) and two levels of *Mesorhizobium* $(R_0=uninoculated and R_1=inoculated)$ inoculants treatment combinations were applied to the plots. The first treatment combination i.e., 17 kg P/ha, 7 kg S/ha and uninoculated is a blanket recommendation of 100 kg NPS fertilizer per hectare for all pulse crop in Ethiopia. The size of each experimental plot was 6 m² (2.4 m x 2.5 m) accommodating eight rows spaced at 30 cm and 10 cm between rows and plants, respectively. The fertilizer sources were urea (46% N), elemental S (95% S), and triple super-phosphate (TSP (20% P). Mesorhizobium ciceri strain CP41 was purchased from Menagesha Biotechnology Private Limited Company, Addis Ababa. This strain has been proven to enhance the nodulation capacity, agronomic, and yield performance of chickpea under wide ecological conditions (Wondwosen et al., 2016; Wondwosen et al., 2017).

Land plowing and leveling was done as per the recommendation for the crop. Seeds were inoculated with *Mesorhizobium* inoculum before sowing following the procedure developed by Wondwosen *et al.* (2016).

The inoculant gently mixed with dry seeds at the rate of 10 g per kg of seed. Inoculation was done just before sowing under shade for avoiding direct sunlight to maintain the viability of cells and allow to air dry for a few minutes and then the inoculated seeds were sown at the recommended rate and spacing to the respective plots. To avoid contamination, plots with un-inoculated seeds were planted first, followed by the inoculated ones. All treatments, except the check plot received equal amount of 19 kg N/ha as a starter.

Data collection

Phenology and growth parameters

Data were collected on plant and plot basis for different agronomic traits. Days to flowering was recorded as the number of days form emergence to when 50% of the plants had flowered in a plot. Days to 90% maturity was the number of days from emergence to the stage when 90% of the plants in a plot have reached physiological maturity. Shoot dry weight was recorded at 50% flowering stage of the plant from ten randomly selected plants during nodule collection time and it was dried at 70 °C in an oven until a constant weight was attained. The observations were recorded in each treatment, in each replication, on five randomly selected plants, excluding border plants for plant height, number of primary branches per plant, number of secondary branches per plant and their mean values were used for statistical analysis.

Nodule number per plant

Nodulation assessment was undertaken at mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to drain water from their surface. Nodules were counted and their average was taken for plots as nodule number/plant. Then after, the nodules were oven-dried at 70°C for 48 h for nodule dry weight determination.

Yield and yield components

Yield and yield components parameters were determined at harvesting time. For the determination of yield components such as number of pods per plant and number of seeds per pod ten randomly picked plants were used. Thousand-grain weight was determined by weighing 1000 randomly selected grains of each plot and weighing with sensitive balance at 10.5% moisture content and it was reported as 1000 grain-weight. Grain and straw yield were recorded from the six central rows of each plot. Total above ground biomass yield was recorded. Grain yield was determined after threshing and adjusting the grain yield at the appropriate moisture level of 10.5%. Straw

yield was calculated by subtracting grain yield from the corresponding total above ground biomass yield. Finally, yield per plot converted to per hectare basis.

Plant tissue analysis

At physiological maturity, net plot samples were collected per plot and partitioned into grain and straw for the determination of N, P and S concentration in the grain and straw. For plant sample analysis, grounded 0.25 g grain and 0.50 g straw samples were taken and digested with a 2:1 mixture of nitric (HNO₃) and per chloric acids (HC1O₄). Phosphorus concentration was analyzed calorimetrically (Morais and Rabelo, 1986). Nitrogen concentration was determined using the Modified micro-Kjeldahl Method (Jackson, 1958). Sulfur concentrations in seed and haulm sub-samples were determined by turbidimetric method using a spectrophotometer by di-acid (HNO₃ and HClO₄) in the ratio of 9:4 for sample digestion (FAO, 2008).

Estimation of Total N, P and S Uptake

Nutrient uptake: Nitrogen, P and S uptake by the grain and straw were determined from the N, P and S content of the respective part by multiplying the grain and straw yield, respectively. Total N, P and S uptake were calculated by adding N, P and S uptake of grain and straw.

Statistical analysis

The collected data were subjected to three factors analysis of variance (ANOVA) to evaluate the main and interaction effect of the factors (phosphorus, sulfur and inoculant) using SAS 9.4 statistical software. Wherever the treatment effects were significant, the mean separation was performed using Duncan's Multiple Range Test (DMRT) at 5% probability level.

Partial Budget Analysis

Yield from experimental plots were adjusted downward by 15%, i.e. 10% for management difference and 5% for plot size differences, to reflect the difference between the experimental yield and the yield that farmers could expect from the same treatment. Accordingly, the mean grain yields for P, S and Mesorhizobium inoculate treatment combinations were subjected to a discrete partial budget analysis using the procedures outlined by CIMMYT (1988). Based on the partial budget procedures, the cost of N was not included because it was applied uniformly to all plots as a starter and was not a variable cost. To estimate economic parameters, the variable cost of P fertilizer (ETB 18.55/kg), sulfur (ETB 180/kg) and Mesorhizobium inoculant (ETB 160/kg) was taken by considering the price at the time of planting (August, 2018). Price of current chickpea grain (ETB 22/kg) and price of straw (ETB 1.3/kg) data were taken from the Office of Trade and Transportation marketing case team of Mortena Jiru district (January to February 2019). The price of phosphorus fertilizer, sulfur and Mesorhizobium

inoculants were taken from Agricultural Inputs Supply Enterprise and Menagesha Biotech Industry PLC, respectively.

RESULTS AND DISCUSSION

Soil physico-chemical pproperties of the study area

The physico-chemical analysis of the experimental soil before planting is presented in Table 1. The laboratory analysis revealed that the particle size distribution in the soil was 12% sand, 14% silt and 74% clay at the experimental site. Hence, the soil texture of the experimental sites was clay. The relatively high clay contents are important to hold residual moisture, which can minimize water stress for the crop. High clay content meets one criterion of Vertisols (clay > 30%) (Asmare et al., 2015). The bulk density of the soil was 1.12 gm/cm³ which approach the ideal value (1.10 g/cm³) for clayey soils for good plant growth (USDA, 2008). The soil pH (H₂O) was 6.41, which the soil pH was under slightly acidic soil condition (Tekalign et al., 1991). Thus, the value was within the optimum range of pH for crop production (6.0 and 8.2) (Horneck et al., 2011).

According to the organic matter (OM) content rating established by Tekalign et al. (1991), the soil had very low OM content (Table 1). The reasons for the very low OM content of the soil could be attributed to intensive cultivation of the land and the total removal of crop residues for animal feed. Moreover, no practice of organic fertilizers addition, such as animal manure and green manure that would have contributed to the soil OM pool in the study area. Similarly, Kiflu and Sheleme (2013) reported that Vertisols of Ethiopia had low soil OM content. The total N content of the soil (0.06%) was rated as low (Tekalign et al., 1991). Total nitrogen found as one of the limited plant nutrient in the study site. As the area receives high rainfall, the N washed out because of runoff problem can be another reason for the decline of the total N in the cropped fields. Other causes of low level of total N might be attributed to its low level of OM content of the study area

The available P content of the soil was rated as low, according to Olsen *et al.* (1954). Low contents of available P are a common characteristic of most of the soils in Ethiopia (Asmare *et al.*, 2015). The available S content is 7.6 mg/kg, which might be regarded as low as per the category suggested by Lewis (1999). This finding is in line with Fanuel *et al.* (2017) who reported that lower available S content in cultivated soils could be linked to lower soil OC, crop uptake and non-use of S fertilizers. Soil fertility map of the Ethiopian soil information system (Etho-SIS) showed that S content of almost all soils of Mortena Jiru district were reported in a very low range (Etho-SIS, 2016). Other author also reported that Vertisols are deficient in S (Hillette *et al.*, 2015).

Table 1. Physical and c	chemical properties of soils of th	ıe
experimental site befor	e sowing	

Soil parameters	Values
Sand (%)	12
Silt (%)	14
Clay (%)	74
Textural class	Clay
Bulk density(cm ³ /gm)	1.12
Soil pH(H ₂ O)	6.41
Organic matter (%)	0.87
Total N (%)	0.06
Available P (mg/kg)	8.65
Available S (mg/kg)	7.6
CEC (cmol(+)/kg soil	44
Exchangeable bases	
Ca (cmol(+)/kg soil	28.4
Mg (cmol(+)/kg soil	12.6
K (cmol(+)/kg soil	1.16
Na (cmol(+)/kg soil	0.76

According to the categories suggested by Hazelton and Murphy (2007), the soils of the experimental site had very high CEC (Table 1). The very high value of CEC is mainly due to high clay content of the experimental site. Although the OM content of the site is low, the amount and type of clay might have been very important in contributing to the CEC values. Moreover, the high CEC values imply that the soil has high buffering capacity against induced chemical changes. These surfaces can attract or adsorb many cations. This is in line with the findings of Asmare et al. (2015) who reported that very high CEC records on Vertisols. As per Hazelton and Murphy (2007) grouping, the soil had very high Ca and Mg and high K and Na content (Table 2). According to Asmare et al. (2015) in Vertisols, the exchangeable sites occupied mainly by Ca and Mg and to a lesser extent by K and Na. Similarly, in the present study, the predominant exchangeable cation, which accounts for more than 66.2% of the exchange complex was Ca⁺², followed by Mg (29.35%), K (2.72%) and Na (1.77%). This might be due to the parent material from which the soils have been developing. This finding is in agreement with to the results of Hillette et al. (2015) who reported that high contents of exchangeable Ca and Mg as a result of which the soil parent material primarily releases divalent cations in higher concentration and are retained for longer periods by the soil colloidal particles because of their higher selectivity coefficient over the monovalent cations.

Phenological parameters

Effects of rhizobia inoculant, P and S application on phenological parameter of chickpea are presented in Table 2. The results of ANOVA revealed significant interaction of rhizobia inoculant, P and S fertilizer application for days to 50% flowering and days to 90% physiological maturity. The interaction effect was not significantly different for both 50% flowering and days to 90% physiological maturity.

Days to 50% flowering

Inoculation of seeds with Mesorhizobium inoculant resulted in the longest days to reach 50% flowering, which was significantly delayed compared to the uninoculated treatment (p< 0.01). The delay in flowering with the Rhizobial inoculation might be due to the fact that inoculation enhanced N fixation and thereby increasing N uptake by plants that might have contributed to improved vegetative growth of chickpea, which might have caused delayed flowering (. The days to flowering of chickpea was significantly earlier with the application of 51 kg P/ha relative to the 34 and 17 kg P/ha (p <0.05). However, no significant difference existed between 34 and 17 kg P/ha (Table 2). This might be due to the fact that the role of P for flowering and seed formation and fastening crop maturity. This finding agrees with that of Verma et al. (2013) who reported that seeds inoculated with rhizobia increased the days to flowering of chickpea. This might be due to the fact that the importance of P for flowering and seed formation and fastening crop maturity. The result is in conformity with that of Asmare et al. (2015) who reported that P plays important role in flowering and seed formation in turn fastening crop flowering. The difference in the maximum and minimum days to attain 50 % flowering was about 2 days only compared with the least rate of S (21kg/ha). This might be due to the role of S, which increases the availability of N and enhances vegetative growth of the crop. In conformity with this result, Reta (2015) who reported that days to flowering was increased with increasing S levels up to 40 kg S/ha.

Days to 90% physiological maturity

Days to physiological maturity was delayed significantly as a result of Rhizobial inoculation compared to un-inoculated treatment (Table 2). This prolonged physiological maturity in response to inoculants might be due to N obtained from biological nitrogen fixation which promotes the vegetative growth. The days to 90% the physiological maturity of chickpea was significantly earlier with the application of 51 kg P/ha than 34 kg P/ha and 17 kg P/ha. This might be due to P required to enhance different plant organs growth (rapid cell division), promote nodulation, and early maturity. This result is in agreement with the finding of Beza (2017) reported that P could also reduce the days to physiological maturity by controlling some key enzyme reactions that involve in hastening crop maturity. The difference in maximum and minimum days to attain 90% physiological maturity of the crop was about 3 days only compared with 21 kg S/ha and 7 kg S/ha application (Table 2). This might be due to the fact that S favorably

influenced the activation of enzymes and chlorophyll synthesis as well as increased carbohydrate metabolism which improved vegetative growth of chickpea rather than early maturity.

Nodulation test

The interaction effect of inoculant and P was significantly influenced the number and dry weight of nodules per plant (Table 3). The highest (47.23) nodule number and nodule dry weight (460 mg) was obtained from the combined application of inoculant with 51 kg P/ ha; while the lowest (32.62) and (370.05 mg) was obtained from 7 kg P/ha without inoculation. This might be due to the vital role of P is required for plant growth, nodule formation and development, and is vital for N2 fixation as well as for the rhizobia bacteria to infect the roots to form nodules. Rhizobia inoculation significantly increased the number and dry weight of nodules per plant mainly because the nitrogenase enzyme present in the bacteria has introduced through infection causes nodule formation. This result is in line with Endalkachew et al. (2018) who observed that the combined application of P with rhizobia inoculant increased the number and dry weight of nodule on chickpea. Moreover, the interaction effect of inoculant and S application was significantly influenced the number and dry weight of nodules per plant in chickpea (Table 6).

The highest (42.82) nodule number and nodule dry weight (450.44 mg) was recorded at inoculant with 21 kg S/ha application while the lowest number (35.60) and nodule dry weight (379.55 mg) nodules were found at the least rate of S (7 kg/ha) without inoculated. The increased number and dry weight of nodules per plant might be due to rhizobia inoculant and S have the role to improve the leghaemoglobin contents of nodular tissue and increase in the nitrogenase activity in turn more number of nodules of large size that ultimately increased the dry weight of nodules. In agreement with this result, Parkash et al. (2017)have observed that the number and dry weight of nodules increased at combined application S and rhizobia inoculation in mung bean.

Growth Parameter

Shoot dry weight

Analysis of variance revealed that there were significant differences among the combined P application with *Rhizobial* inoculant treatments for shoot dry weight (Table 3). The highest mean value of shoot dry weight was obtained from the combined application of 51 kg P/ha (13.91 g) with inoculant while the lowest value (7.17 g) was recorded from the least rate of P (17 kg/ha) application without inoculant. This might be mainly due to the fact that P application favored plants establish a well-developed root system with enhanced nitrogen-fixing capacity and nodule

formation that resulted in the better availability of N, better growth and development of plants that ultimately increased all the growth attributes, including shoot dry weight of plants. This result is in line with Singh et al. (2018) who reported that the application of P at the rate of 60 kg/ha with rhizobia inoculation significantly increased shoots dry weight of chickpea over the control and un-inoculated treatment. Moreover, there was a highly positive interaction between S application and inoculant for shoot dry weight (Table 6). The highest value (13.08 g) was recorded from the application of 21 kg S/ha with inoculant; while the lowest value (8.40 g) was obtained from the application of 7 kg/ha without inoculation. In agreement with this result, Beza (2017) reported that the combined application of S fertilization with rhizobia increased shoot dry weight on chickpea.

Table 2: Effects of *Mesorhizobium* inoculant, P, and S application on phenological parameters of chickpea

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Treatments	Days to	Days to 90%		
	50%	Physiological		
	flowering	maturity		
Inoculant (R)				
Un-inoculated	48.85 ^b	134.48 ^b		
Inoculated	49.85 ^a	135.33ª		
P value	**	*		
Phosphorus (kg/	/ha)			
17	49.83a	135.27 ^a		
34	49.61 ^a	135.16 ^a		
51	48.83 ^b	134.27 ^b		
P value	*	*		
Sulfur (kg/ha)				
7	48.50 ^c	133.50 ^c		
14	49.24 ^b	134.66 ^b		
21	50.50 ^a	136.55ª		
P value	**	***		
P*S	NS	NS		
P*R	NS	NS		
S*R	NS	NS		
P*S*R	NS	NS		
CV (%)	2.1	1.2		

Main effect means within a columns followed by the different letter(s) are significantly different from each other at P >0.05; NS = non-significant; * = significant at P \leq 0.05; ** = significant at P \leq 0.01; *** = significant at P \leq 0.001. CV= Coefficient of variation, P= phosphorus, S= sulfur and R= *Mesorhizobium* inoculant.

Plant height

Plant height of chickpea increased as a result of the combined application of P fertilizer with *Rhizobial* inoculant (Table 3). The longest (54.03 cm) and the shortest (43.52 cm) plant height was produced in response to 51 kg P/ha application with inoculant and 17 kg P/ha without inoculant. In agreement with this result, Singh *et al.* (2018) reported the application of P

and rhizobia inoculant increased plant height of chickpea. The analysis of variance also showed that the combined application of P and S significantly affected plant height (Table 4). The longest (54.82 cm) and the shortest (43.65 cm) plant heights were obtained at 51 kg P/ha with 21 kg S/ha, and (17 kg P/ha) with (7 kg S/ha) application, respectively. This might be due to the fact that S increased the activity of rhizobia and nutrient (N and P) availability to the crop and effect of P on meristematic cell activities that can play a vital role in vegetative growth that might have ultimately increased the plant height in chickpea. This finding agrees with Parkash *et al.* (2017) who reported that the combined application of P and S increased the plant height of chickpea.

Table 3. Phosphorus x *Mesorhizobium* inoculant interactions for nodulation, growth, yield and yield components and total N and P uptake in chickpea.

Treatment NNP	NINID	NDW	SDP	PH	NPP	TGW	NCP	GY	SY(kg/ha)	TNU	TPU
	ININI	(mg)	(g)	(cm)	INFT	(g)	NGI	(kg/ha)		(kg/ha)	(kg/ha)
P1R0	32.62 ^d	370.55 ^d	7.17e	43.52^{f}	43.2e	267.7d	1.18 ^d	1406 ^f	1161.67d	42.03d	8.38d
P1R1	34.20 ^{cd}	388.77 ^c	8.92d	45.32d	44.8 ^d	272.5 ^{dc}	1.27c	1410.11 ^d	1200 ^{cd}	45.25 ^{cd}	8.83cd
P2R0	36.10 ^c	378.88 ^c	8.77d	44.77df	48.7c	290.5bc	1.23 ^{cd}	1601.44 ^c	1216.11 ^c	46.58c	10.16 ^c
P2R1	42.22 ^b	416.77 ^b	11.94 ^b	50.53 ^b	56.8 ^b	298.8c	1.44 ^b	1731.33 ^{bc}	1320.56bc	51.92 ^b	11.84 ^{cb}
P3R0	41.63 ^{bc}	408.66 ^b	10.76 ^c	47.99 ^c	55 ^{bc}	315.2 ^b	1.28 ^c	1734.89 ^b	1329.33 ^b	53.49 ^b	11.54 ^b
P3R1	47.23ª	460.00 ^a	13.91ª	54.03 ^a	66.3ª	339.5ª	1.56 ^a	2202.33a	1481.36 ^a	71.41ª	15.41ª
PValue	**	*	**	*	**	*	*	**	*	*	*
CV (%)	5.16	6.6	6.9	5	7.17	6.12	6.93	7.1	8.95	12.47	10.84

Means followed by the same letter within a column are not significantly different at 5% level of significance by DMRT. *, **, ***, and NS showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively. NNP= nodule number per plant, NDWP= nodule dry weight per plant, SDP= shoot dry weight per plant, PH= plant height, NPP= number of pod per plant, TGW= thousand grain weight, NGP= number of grain per pod, GY= grain yield, ST= straw yield, TNU= total nitrogen uptake and TPU= total phosphorus uptake.

Table 4. Phosphorus and Sulfur levels interactions for growth, yield and yield components and total N and P uptake in chickpea

				TGW	GY		TNU	TPU	TSU
Treatment	PH (cm)	NPBP	NPP	(gm)	(kg/ha)	SY (kg/ha)	(kg/ha)	(kg/ha)	(kg/ha)
P1S1	43.65 ^f	2.02 ^d	40.2 ^d	266.6 ^e	1323.33e	1140.50^{f}	39.62 ^f	7.72 ^f	8.99 ^d
P1S2	43.85 ^d	2.13bc	44.3cd	269.3 ^{de}	1370 ^{de}	1161.14 ^d	41.25e	8.5 ^{ef}	10.79 ^{cd}
P1S3	45.68 ^{cd}	2.25 ^b	47.8°	274.0 ^{cde}	1528.83c	1241.23 ^{cd}	45.55 ^{de}	9.56 ^{de}	13.25 ^{cd}
P2S1	44.65 ^d	2.14 ^{bc}	47.2 ^c	283.6 ^{cd}	1492.33 ^{cd}	1142.67 ^d	43.41 ^d	9.43 ^{de}	10.74^{cd}
P2S2	48.96 ^b	2.17 ^c	56.9 ^b	289.0 ^c	1707.50 ^b	1296 ^{bc}	50.93°	11.03c	11.65 ^{cd}
P2S3	49.33 ^b	2.28 ^b	55.3 ^b	311.5 ^b	1799.33 ^b	1379.83 ^b	53.41 ^{bc}	12.54 ^b	15.36 ^b
P3S1	48.18^{bc}	2.09bc	55.6 ^b	310.3 ^b	1490.67 ^{cd}	1189.13 ^{cd}	45.93cd	10.45 ^{cd}	11.03 ^{cd}
P3S2	50.03 ^b	2.27 ^b	59.6 ^b	312.5 ^b	1836.17 ^b	1373.83 ^b	55.45 ^b	12.24 ^b	13.76 ^{bc}
P3S3	54.82 ^a	2.78 ^a	67.8ª	359.3ª	1951.33ª	1402.50a	60.98 ^a	14.72 ^a	21.43ª
P value	*	**	*	**	*	*	***	*	*
CV (%)	5	8.02	7.17	6.12	7.1	8.95	12.47	10.84	18.95

Means followed by the same letter within a column are not significantly different at 5% level of significance by DMRT. *, **, ***, and NS shows significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively. PH= plant height, NBPP= number of primary branches per plant, NPP= number of pod per plant, TGW= thousand grain weight, GY= grain yield, ST= straw yield, TNU= total nitrogen uptake, TPU= total phosphorus uptake and TSU= total sulfur uptake.

Number of branches per plant

The interaction effects of P and S significantly influenced the number of primary branches on chickpea (Table 3). The highest values (2.78) of primary branches obtained from the combined application of 51kg P/ha and 21kg S/ha. Similarly, the least rates of P (17 kg/ha) and S (7 kg/ha) produced the lowest (2.02)

number of primary branches per plant. This might be due to the role of P and S to facilitate plant roots development and enhanced atmospheric nitrogen fixation, which promoted the development of the vegetative parts which includes branches. This result is in line with, Sharma *et al.* (2015) who reported that the combined application of P and S increased the number of primary branches on chickpea. The three-way interaction effects of inoculant, P and S application significantly affected the number of secondary branches per plant (Table 5). The data showed that the highest value (18.6) secondary branches per plant was obtained from the combined application of inoculant with P (51 kg/ha) and S (21 kg/ha) while the lowest value (8.46) was obtained from non inoculated with the least rate of P (17 kg/ha) and S (kg/ha). This finding agrees with Sharma et al. (2015) who reported that the application of different doses of sulfur and phosphorus with inoculant significantly increased the number of branches in chickpea.

Table 5: Interaction effects of *Mesorhizobium* inoculant, P, and S application on number secondary branches of chickpea

		Secondary branches per					
Trea	atments	plant					
		Р	P levels kg/ha				
	S (kg/ha)	17	34	51			
R_1	7	13.2 ^d	11.8e-g	15.73 ^{bc}			
	14	12.33d-f	13.06 ^{de}	17.2 ^b			
	21	13.4 ^d	15.06 ^c	18.6 ^a			
	7	8.46j	9.8 ^{hi}	11gh			
R_0	14	9.48^{i}	10.8gh	10^{hi}			
	21	10.93gh	11.33 ^{fg}	12.66 ^{de}			
P value			*				
CV (%)			5.7				

Means within a columns followed by the different letter(s) are significantly different from each other at P > 0.05; ns = non-significant; * = significant at P \leq 0.05; *** = significant at P \leq 0.001. CV= Coefficient of variation, P= phosphorus, S= Sulfur R=*Mesorhizobium* inoculant.

Grain yield and yield components

Number of pods per plant

The interaction effects of P and S also significantly influenced the number of pods per plant (Table 4). The highest (67.78) number of pods per plant was obtained from the combined application of P (51 kg/ha) with S (21 kg/ha); while the lowest (40.12) was recorded from the least rates of P and S i.e., 17 kg/ha and 7 kg/ha, respectively. This might be due to the fact that P and S have vital role for improved availability of N from BNF, which could have facilitated the production of branches and plant height and this in turn have contributed to the production of a higher number of pods per plant. Furthermore, the application of P and S promotes both vegetative and reproductive development, thereby improving the photosynthetic efficiency and partitioning of carbohydrate that in turn might have increased the number of pods per plant. This result is in line with Sharma et al. (2015) who reported that the combined application of P and S increased number of pods per plant in chickpea.

The interaction of P and inoculant significantly affected the number of pods per plant in chickpea (Table 3). Application of 51 kg P/ha along with seed inoculated by Rhizobial gave the highest number of pods (66.32), while the lowest number of pods per plant (43.20) was obtained from the un-inoculated seeds with the least rate of P (17 kg/ha). Increase in the number of pods per plant with the application of P and seed inoculation might be due to the supply of N through biological nitrogen fixation and the cumulative effect of P in the processes of cell division and balanced nutrition that in turn might have played an important role in the growth and assimilate accumulation, thereby improving the reproductive performance of the plants, which ultimately increased pods per plant. The current result is in line with, Sharma et al. (2015) who reported that rhizobia inoculant in association with P application increased number of pods per plant in chickpea.

Thousand grains weight

The analysis of variance revealed significant interaction of P and S application for thousand-grain weight in chickpea (Table 4). The highest (359.33 g) and the lowest (266.67 g) thousand-grain weight was recorded at the combine application of 51 kg P/ha with 21 kg S/ha and 17 kg P/ha with 7 kg S/ha, respectively. This finding agrees with Beza (2017) who reported that the combined application of P and S increased thousandgrain weight of chickpea.

Moreover, the ANOVA also showed that thousand grains weight significantly affected by the combined application of P and *Rhizobial* inoculant (Table 3). While comparing the interaction effects of inoculant and P, the highest (339.55 g) and the lowest (267.77 g) thousand-grain weight was obtained at the rate of 51 kg P/ha with inoculant and 17 kg P/ha without inoculant (uninoculated) application.

Table 6. Sulfur x *Mesorhizobium* inoculant levels interactions for inoculation, growth and yield in chickpea

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Treatment	NNP	NDP	SDWP	GY
S_1R_0	35.60 ^d	379.55d	8.40 ^d	1430.11^{f}
S_1R_1	38.28 ^c	405.22 ^b	10.56bc	1442.11 ^d
S_2R_0	34.75 ^{dc}	385.44 ^c	8.69 ^{cd}	1579.33 ^c
S_2R_1	42.62 ^b	409.88 ^b	11.13 ^b	1696.44 ^{bc}
S_3R_0	39.93 ^{bc}	393.11 ^{bc}	9.60 ^c	1737 ^b
S_3R_1	42.82 ^a	450.44a	13.08a	1950a
P Value	***	*	*	*
CV (%)	5.16	6.6	6.9	7.1

Means followed by the same letter within a column are not significantly different at 5% level of significance by DMRT. *, **, ***, and NS showed significant differences at 0.05, 0.01, 0.001 probability levels and non-significant differences, respectively. NNP= nodule number per plant, NDP= nodule dry weight per plant, SDWP= shoot dry weight per plant and GY= grain yield.

Number of grains per pod

Significant differences were found for number of grains per pod with a combined application of 51 kg P with *Rhizobia* inoculant than un-inoculated (Table 3). The highest (1.56) and the lowest (1.18) number of grains per pod was recorded at 51 kg P/ha with the inoculant and 17 kg P/ha without inoculant, respectively. The result of the present study was in conformity with Endalkachew *et al.* (2018), who reported that the combined application of P with inoculant significantly increased the number of grains per pod in chickpea.

Grain yield

The interaction effect of P and S significantly influenced grain yield in chickpea at P < 0.05 (Table 4). Phosphorus application at the rate of 51 kg/ha and S application at the rate of 21 kg/ha resulted in the highest grain yield (1951.33 kg/ha); while the minimum grain yield (1323.33 kg/ha) was recorded from the application of P at the rate of 17 kg/ha and S at the rate of 7 kg/ha, but statistically at par with P rate of 17 and 14 kg/ha. This might be due to the synergistic effect of P and S that might have enhanced the utilization of high quantities of nutrients through their well-developed root system and nodules, which might have resulted in better vegetative and reproductive growth and development that leads to increased grain yield. This result is in line with; Parkash et.al (2017) who reported that the combined application of P and S might have led to increased vegetative growth, nodulation and yield component, which in turn resulted in grain yield of chickpea. Moreover, P application with inoculated seeds significantly affected grain yield of chickpea (Table 3). Inoculated seeds with 51 kg P/ha application produced the highest grain yield (2202.33 kg/ha); while the minimum grain yield (1406 kg/ha) was recorded by un-inoculated seeds with a lower rate of P (17 kg/ha) and inoculated seeds with 17 kg P/ha (Table 10). This was mainly due to the fact that better availability of N and P caused well-developed root system having higher N fixing capacity resulting in better growth and development of plants that might have eventually led to increased yield. This result is in line with Endalkachew et al. (2018), who reported that the combined application of Mesorhizobium inoculant with P significantly increased grain yield in chickpea.

In addition, the interaction effects of S application and inoculant was significant for grain yield (Table 6). The application of S at the rate of (21 kg/ha) with an inoculant results in the highest grain yield (1950 kg/ha); while the lowest grain yield (1430.11 kg/ha) was obtained from the lowest rate (7 kg S/ha) with and without inoculant. The increased in grain yield of chickpea in response to *Mesorhizobium* inoculant might be due to sufficient N supply mainly from biological nitrogen fixation and S also necessary for enzymatic action, chlorophyll formation, synthesis of certain amino acids and vitamins which increased vegetative and reproductive growth that in turn increased grain yield. This result is in line with Beza (2017) who reported that the combined application S with rhizobia inoculant increases grain yield of chickpea.

Straw yield

The straw yield was significantly affected by the interaction effect of P and S application (Table 4). While comparing the P rates, the highest (1355.17 kg/ha) and the lowest (1272.83 and 1180.83 kg/ha) straw yield were obtained from the application of 51 kg, 34kg and 17 kg P/ha, respectively. In conformity with this result, Sharma et al. (2015) reported that the combined application of P and S significantly increased the straw yield on chickpea. Moreover, the analysis of variance revealed that straw yield significantly affected by the combined application of P and inoculant (Table 3). Comparing the effect of the S rates on straw yield, the application of 21 kg, 14 kg and 7 kg S/ha gave 1374.44, 1276.94 and 1157.44 kg/ha, respectively. This might be due to the fact that P and rhizobia inoculant in legume plants significantly increased nodulation and improves vegetative growth and development of plants, which leads to increased straw yield. In line with this result, Beza (2017) also reported that the straw yield increased the combined application of P fertilizer with inoculant in chickpea plant.

Total nitrogen uptake

The combined application of P fertilizer with inoculant significantly affected the total N uptake on chickpea (Table 3). The maximum (71.41 kg/ha) and the minimum (42.03 kg/ha) TNU was recorded at 51 kg P/ha with inoculant and 17 kg P/ha without inoculate, respectively. This might be due to rhizobia and P enhanced the nodule formation, stimulate N fixation and increase the availability of N for chickpea plants that results increased the uptake of N by straw and grain that ultimately increased total N uptake. In addition, total N uptake of chickpea increased significantly due to increased levels of P might be due to the well-developed root system, which helps in increased nitrogen fixation and its availability to plant along with other nutrients that in turn results to increases total N uptake. In agreement with the present study Endalkachew et al. (2018) reported that a significant increase in total N uptake due to Mesorhizobium inoculant with P application in chickpea. The result indicated that the P and S application has shown a tendency to be relatively more important in affecting total N uptake (Table 4). The highest total N uptake (60.98 kg/ha) was recorded at the combined application of 51 kg P/ha along with 21kg S/ha, while the lowest (39.62 kg/ha) was obtained at 17 kg P/ha with 7 kg S/ha application. This might be due to the effect of P application on N fixation that affect host plant growth, whereas S was directly involved in N fixation that increased the availability of N which ultimately increased the uptake of N by grain and straw which ultimately increased total N uptake. This result is in line with Sharma et al. (2015) who reported that total N uptake in chickpea increased significantly with the application of both P and S.

Total phosphorus uptake

Total P uptake of chickpea was significantly (P<0.001) affected by the combined application of P and Mesorhizobium inoculant (Table 3). The highest (15.41 kg/ha) and the lowest (8.38 kg/ha) total P uptake was obtained at 51 kg P/ha with inoculant and 17 kg P/ha without inoculant, respectively. The significant increasing trend of total P uptake might be due to increased concentration of P in soil solution with increasing P application and increase in biological activity by inoculant. In addition, the higher P uptake due to rhizobia inoculation might be attributed to the fact that some isolates of rhizobia have the ability to solubilize unavailable phosphorus components to available form. The result is in line with Sharma et al. (2015) who reported that the application of P with rhizobia inoculant increase the total P uptake on chickpea.

Moreover, the total P uptake was significantly affected by the combined application of P and S (Table 4). At the application P at the rate of 51 kg/ha with application S at a rate of 21 kg/ha was obtained maximum total P uptake (14.72 kg/ha) while application P at a rate of 17 kg/ha with application S at a rate 7 kg/ha was obtained minimum total P uptake (7.72 kg/ha). This might be due to the fact that the available P in soil increased and its absorption by the plant also improves with the addition of S and P. Furthermore, it might be due to the fact that S has the important role to release of more soil P from the adsorption site thereby ion exchange synergistically. This result is in line with Singh et al. (2018) who reported that application of 60 kg P/ha with 40 kg S/ha significantly increased total P uptake on chickpea.

Total sulfur uptake

The combined application of P and S fertilizers significantly (p<0.05) affected the total S uptake (Table 4). The highest total S uptake 21.45 kg/ha was recorded at the application of P (51 kg/ha) with S (21 kg/ha)

21

fertilizer while the lowest (8.99 kg/ha) recorded at the application of P (17 kg/ha) with S (7 kg/ha). This result might be due to the fact that the application of P and S enhanced the availability of P and S in the soil solution which ultimately increased the uptake of P and S by seed and straw as a result increased the total S uptake.

Partial Budget Analysis

Partial budget analysis allows assessing the impact of a change in the production system on a farmer's net income without knowing all his costs of production. Data presented in Table 7 indicated the economic analysis of chickpea as affected by the effects of *Mesorhizobium* inoculant, P and S fertilizer rate. It is clear from the budget summary of economic analysis, the highest net benefit (38,699.9 ET Birr/ha) was obtained from seeds inoculation with 51 kg P/ha and 7 kg S/ha, followed by 51 kg P/ha with 21 kg S/ha (32,175.9 ET Birr/ha), 34 kg P/ha with 14 kg S/ha (27305.7ET Birr/ha) and 17 kg P/ha with 7 kg S/ha (25209.8 ET Birr/ha.

According to dominance, analysis as indicated on (Table 7) most of the treatments dominated by the highest net benefit treatments hence, eliminated for further economic analysis. Data from Table 21 clearly showed that the non-dominated treatments associated with marginal rate of return (MRR) are greater than 100%. This implies that the four non-dominated treatments were economically feasible alternative to the other dominated treatments. The highest MRR of 7.9 % obtained from combined application of was Mesorhizobium inoculate, 51 kg P/ha and 21 kg P/ha. This implies that for 1.00-Birr investment in chickpea production, the producer can get 7.9 ET Birr. The second the highest MRR of 5.8 % was recorded from companied application of 34 kg P/ha with 14 S kg/ha. The second MRR obtained from P with S fertilizer rate was decreased. Generally, treatment combination of Mesorhizobium inoculant with 51 kg P/ha and 21 kg S/ha gave better MRR value relative to the other nondominated treatments and profitability can be optimized by using this treatment. From this finding, it observed that inoculant was crucial for chickpea production with P and S fertilizer application.

 Table 7. Partial budget of un-dominance

	Treatment		ADJGY	ADJSY	TGB	TVC	NB	MRR
P (kg/ha)	S (kg/ha)	R (kg/ha)	(kg/ha)	(kg/ha)				(%)
17	21	0	1275.9	1061.9	29449.2	4239.4	25209.8	1.7
34	14	0	1401.9	1064.5	32226.4	4920.7	27305.7	2.8
51	21	0	1726.4	1213.2	39556.9	7381.0	32175.9	5.8
51	21	0.5	2017.6	1341.0	46130.9	7431.0	38699.9	7.9

P= Phosphorous, S= Sulfur, R=*Mesorhizobium* inoculant, ADJGY=Adjusted grain yield, ADJSY= Adjusted straw yield, TGB= Total gross benefit, TVC= Total variable cost, NB= Net benefit, MRR= Marginal rate of return.

CONCLUSION

Understanding chickpea response to biofertilizer inoculation and fertilizer application rates (P and S) is an important agronomic management consideration for chickpea production that can have a significant effect on grain yield. Mesorhizobium inoculation and application of phosphorus and sulfur improved nodulation parameters (nodule number and nodule dry weight), plant growth parameters (plant height and number of branches) and yield and yield components (number of pod per plant, thousand-grain weight, grain yield and straw yield) and nutrient uptake (N uptake and P uptake) tested in this study. Better results were recorded in plots supplied with the inoculant, and combination of phosphorus and sulfur application at the rate of 51 kg/ha and 21 kg/ha, respectively. Significant interactive effects exhibited by inoculating the soil with Mesorhizobium, and supplementing it with phosphorus and sulfur suggest the need of this technology in the study area. Thus, biofertilizer and appropriate fertilizer-P and S management will ensure optimal agronomic and economic returns in the study area, although the experiment need to be repeated in more locations and season to draw a conclusive recommendation.

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