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Nutrient Utilization and Yield Response of Lentil (*Lens culinaris* Medikus) to *Rhizobium* Inoculant and Sulphur Fertilization

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Abstract: Lentil (Lens culinaris Medikus) is a major food crop in Ethiopian. It is affordable protein source and important in sustaining soil fertility through nitrogen fixation. However, its current national productivity of 1.46 t ha^{-1} is lower than its potential due to inadequate agronomic management practices, nutrient imbalance and lack of indigenous or commercial Rhizobium strains of lentil. Therefore, the field experiment was conducted at Ada'a district under rain-fed condition during 2016/17 main cropping season to assess the role of S and Rhizobium inoculant on nodulation, nutrient utilization and yield response of lentil. The experiment consisted of three levels of S (0, 20 and 40 kg ha^{-1}) and Rhizobium inoculant (un-inoculated and inoculated) in a factorial combination using Alemaya lentil variety as a test crop. The experiment was conducted using randomized complete block design with three replications. The result showed the interaction of sulphur fertilization and Rhizobium inoculant were significant on days to flowering, number of nodules plant^{-1}, nodule dry weight plant^{-1}, number of seeds pod^{-1}, aboveground dry biomass, seed yield, seed S uptake, haulm S uptake, total S uptake, sulphur agronomic and recovery efficiency as well as sulphur harvest index. Application of 40 kg S ha^{-1} without Rhizobium inoculant led to produce the highest seed yield (2.27 t ha^{-1}) and delayed days to flowering (46 days) of lentil whereas, the highest nodule dry weight plant^{-1} (1.1mg) and sulphur harvest index (17.68%) were obtained at the rate of 40 kg S ha^{-1}under Rhizobium inoculations. On the other hand the maximum number of nodule plant^{-1} (15.60), number of seed pod^{-1} (1.99), total aboveground dry biomass (8.22 t ha^{-1}), Sulphur agronomic efficiency (32.11kg ha^{-1}), sulphur recovery efficiency (66.00 kg ha^{-1}) were obtained in response to sulphur application at 20 kg ha^{-1} under Rhizobium inoculations. Moreover, Rhizobium inoculation without S fertilization gave the highest seed (3.88kg ha^{-1}), haulm (23.33kg ha^{-1}) and total S uptake (24.89 kg ha^{-1}). Rhizobium inoculation without S application had high net benefit, relatively low variable cost with an acceptable and maximum MRR for lentil production in Ada'a district. However, since the experiment was conducted only for one season and one site, repeating the trial at different sites as well as in the same trial site would be important in order to draw sound recommendation.

Keywords: Nutrient Utilization, Rhizobium Inoculants, Sulphur

1. Introduction

Lentil (*Lens culinaris* Medikus) is an important dietary source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds, as well as diverse non-nutritional components like protease inhibitors, tannins, -galactoside oligosaccharides and phytic acid [1]. It contains 0.7- 4.3g fat, 43.4-69.9 g carbohydrates, 5.0-26.9 g fibers and 2.2-4.2 g ash, while energy and total nitrogen are 1483-2010 KJ and 3.72-4.88 g per 100 grams of seed, respectively [2]. In Ethiopia, lentil is mainly consumed as a traditional main dish to accompany *injera* and major source of protein in the

Ethiopian diet, which are consumed during fasting and non-fasting days [3]. It is usually eaten fried, roasted and whole boiled, or split in the form of stews, vegetable soups mixed with other beans, or sometimes prepared as "*shiro*" and "*Azifa*" [4].

Despite efforts to boost productivity of lentil in Ethiopia the past decade, the national average productivity is still low (1.46 t ha^{-1}) as compared to the potential yield of lentil [5]. However, improved varieties can yield 1.4-5 t ha^{-1} under research fields and 0.9-3 t ha^{-1} under farmer's fields with full use of agronomic packages [6]. This huge gap of productivity difference is emanated from variability of crop husbandry practices such as soil, crop, fertilizer and water management. In addition to this, lack of maintaining soil fertility and use of plant nutrient in balanced amount is one of the key components to decrease crop production and productivity [7].

Sulphur is the key component of balanced nutrient application for higher yields and superior quality produce [8]. Sulphur is the 3rd limiting nutrient next to N and P in highly weathered soils in tropics [9]. Ethiopia is also one of the tropical countries in which S deficiency occurs. The central highlands of Ethiopia is an area in which large crop production is being carried out and characterized by high use of inputs like DAP and UREA. Because of this, S fertilization has not been part of fertilizer recommendation in Ethiopia for a long time. Furthermore, S deficiency could occur due to intensive cultivation, use of high yielding cultivars, accelerated rate of soil erosion and sulphur dioxide emissions in industrial areas [10]. In the future, this situation may be aggravated unless attention is given to reverse it. To this end, it is the right time that the response of lentil to S fertilization is examined in this area.

Rhizobium inoculants offer a new eco-friendly technology which would overcome shortcomings of the conventional chemical based farming and showed positive influence on both soil sustainability and plant growth. They gradually improve soil fertility by fixing atmospheric nitrogen. They also increase the phosphorous content of the soil, restoring depleted nutrients of the soil and improve plant root proliferation [11]. Moreover to these advantages, *Rhizobium* inoculants can decrease the dose of chemical fertilizers. It results in reduced cost of fertilization and it help in increasing the crop yield by 10-25% [11].

Rhizobium inoculation and chemical fertilization significantly increased fat, fiber and protein content of seed in lentil [12]. Proper management coupled with the use of bio-fertilizers not only improves productivity but also helps to bring a large area until lentil cultivation in different cropping systems [13]. Although some yield response with application of sulphur along with *Rhizobium* inoculants have been reported in different countries. So far, no works have been conducted on determination of optimum sulphur fertilizer rate for production of lentil in Ethiopia. Therefore, there is a need to acquire of information on influences of sulphur along with *Rhizobium* inoculants on nutrient utilization, yield and yield component of lentil. Of these, the present study has been undertaken to examine the effect of different levels of sulphur and assess their best combination with *Rhizobium* inoculants on yield, yield component and nutrient utilization of lentil.

2. Materials and Methods

2.1. Description of Experimental Site

The experiment was conducted on farm field at Denkaka kebele in Ada'a district, central highlands of Ethiopia. Denkaka is located 60 km East of Addis Ababa and its geographical extent ranges from 08°45' to 08°46'N and 38°46' to 39°01' E with an altitude 1850 m above sea level. The soil textural class of the experimental site is clay and having the Haplic, Andosol, Vitric Andosol and Vertisol [14]. Soil samples were collected from the experimental field before planting to have an idea about the soil fertility status as indicated in (Table 1).

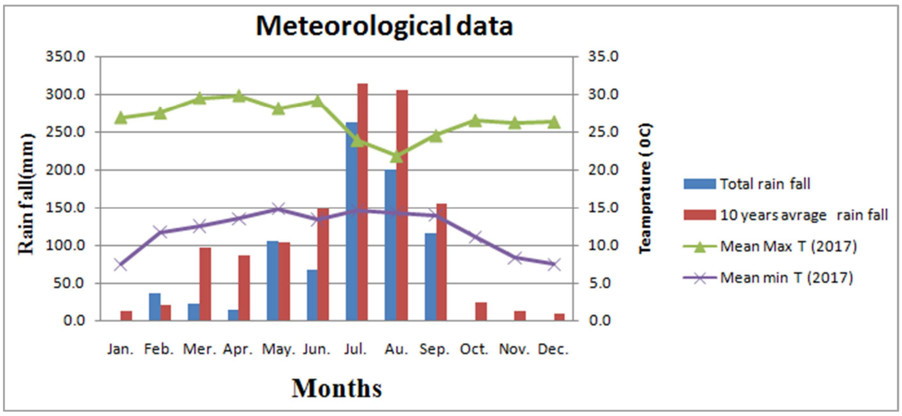

Figure 1. *Monthly mean min and max temperature (°C) and 10 years average and total rainfall (mm) data of the experimental station (in 2016/17) cropping season.*

Table 1. *Chemical and physical soil analysis of field site.*

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH	OM (%)	Av. P (mg. kg-1)	Av. S (mg. kg-1)	CEC (cmol (+) kg-1)	TN (%)
0-20	44	32	24	6.77	1.40	6.18	5.40	35.94	0.09

The data of climatic parameters such as rainfall, maximum and minimum temperature were recorded at meteorological observatory; main agriculture research station, Debre Zeit during cropping period of the experimental year 2017 and mean of past 10 years (2007-2016) are presented in (Figure 1). The area received an annual rainfall of 824.6 mm during the cropping season (January-December, 2017) which was higher than the mean annual rainfall (788.5mm) of the ten years. Mean maximum and minimum temperatures recorded at the station during the season were 25.3 and 13.5°C, respectively. The area received high amount of rainfall from May to September months of the cropping season in which the highest amount (262.3 mm) was obtained in July followed by August (200.2 mm) and nil.

2.2. Treatments and Experimental Design

The experiment was laid out in randomized complete block design with factorial arrangement of treatment combinations and replicated three times. Each replication consisted of six treatment combinations and the total numbers of plots were eighteen. Three levels of S (0, 20 and 40 kg ha^{-1}) and two levels of *Rhizobium* inoculants (un-inoculated and inoculated) treatment combinations were applied to the plots. The size of each experimental plot was 4.2 m^2 (1.4 m × 3 m) accommodating seven rows of plants spaced at 20 cm between rows. A spacing of 50 cm between the plots and 1m between blocks were kept. The outer most rows of both sides were considered as boarder. Second row at both side of each plot was used as sampling row for nodulation study. The other rows were kept for final sampling. Hence, three rows of 2.80 m length (leaving 0.20 m at both sides of plot) were regarded as net plot (0.60 m x 2.80 m = 1.68 m^2).

2.3. Experimental Materials and Procedures

A cleaned seed of Alemaya lentil variety was used for a test crop. The field was prepared with conventional methods using oxen plough. Planting was done in fourth week of July 2017 by drilling seeds per row and thinned at 2 cm apart between plants after successful establishment was assured. The sulphur fertilizer rate was calculated and applied per plots. Source of S was potassium sulphate (K_2SO_4; 51% K_2O & 18% S). Potassium chloride fertilizer was also added to adjust the amount of potassium in plots and both nutrients were obtained from Agricultural inputs supply enterprise. *Rhizobium* inoculant (Et 600) a commercial inoculant, was obtained from Menagesha Biotech Industry PLC in Addis Ababa. Recommended dose of nitrogen was applied equally to all treatments as a starter 30 kg ha^{-1} [15]. All necessary agronomic practices as required by the crop were carried out as per the research recommendation for lentil production.

2.4. Plant Tissue Sampling and Analysis

At harvesting time, five randomly selected plants were harvested from three central rows and partitioned into seed and haulm. Each sample was separately oven dried at 70°C for 24 hrs and ground to pass 1 mm sieve and saved for tissue analysis of seed and haulm. Then sulphur concentrations in seed and haulm sub-samples were determined by turbidimetric method using a spectrophotometer by di-acid (HNO_3 and $HClO_4$) in the ratio of 9:.4 for sample digestion [16]. This concentration of S was used to compute the S uptake which was calculated by multiplying seed and haulm yields on hectare basis with the respective S content in percentage for each plot. Total S uptake was calculated as the sum of seed S uptake and haulm S uptake [17].

2.5. Sulphur Use Efficiency and Harvest Index

Based on the laboratory results of plant tissue analysis; agronomic and recovery efficiency were computed according to the formula described by a study [18] and nutrient harvest index was computed according to the formula described by a study [19]. Sulphur agronomic efficiency (kg kg^{-1}): is defined as the quantity of seed yield per unit of nutrient applied.

$$SAE = \frac{Sf - Su}{Na} \quad (1)$$

Where Sf is the seed yield of the fertilized plot (kg), SU is the seed yield of the unfertilized plot (kg), and Na is the quantity of S applied (kg) and SAE is sulphur agronomic use efficiency. Sulphur recovery efficiency is defined as the quantity of nutrient uptake per unit of nutrient applied.

$$SRE = \frac{Nf - Nu}{Na} * 100 \quad (2)$$

Where Nf is the nutrient uptake (seed plus haulm) of the fertilized plot (kg), Nu is the nutrient uptake (seed plus haulm) of the unfertilized plot (kg) and Na is the quantity of nutrient applied (kg) and SRE is sulphur recovery use efficiency. Sulphur harvest index (SHI) was calculated with the help of the following formula.

$$SHI = \frac{\text{S uptake by seed}}{\text{S uptake by seed+haulm}} * 100 \quad (3)$$

2.6. Data Collected

Phenological, nodule and yield parameters following the standard procedure for the crop such as days to flowering, number of nodules $plant^{-1}$, nodule dry weight $plant^{-1}$ (mg), number of seeds pod^{-1}, total aboveground biomass yield (t ha^{-1}) and seed yield (t ha^{-1}) were collected.

2.7. Statistical Data Analysis

Statistical analysis of the data collected from the field and laboratory were analyzed by SAS version 9.3 statistical software using linear model procedure after checking the compliance of the data with the assumptions of the statistical test [20]. Comparisons among treatment means with significant difference for measured and scored characters were done using least significance difference (LSD) at 5% probability level.

2.8. 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 S and *Rhizobium* inoculation treatment combinations were subjected to a discrete partial budget analysis using the procedures outlined by [21]. To estimate economic parameters, the variable cost of potassium sulphate (ETB 26.9 kg^{-1}), *Rhizobium* inoculants (ETB 3.2 kg^{-1}) were recorded at time of planting. Price of current lentil grain (ETB 21 kg^{-1}) and price of haulm (birr 2.45 kg^{-1}) data were taken from Office of Trade and Transportation marketing case team of Debre Zeit district (January to February 2018). The price of phosphorus fertilizer, potassium sulphate and *Rhizobium* inoculants were taken from Agricultural Inputs Supply Enterprise and Menagesha Biotech Industry PLC at the time of planting, respectively.

3. Results and Discussions

3.1. Days to Flowering

The main effect of *Rhizobium* inoculant was not significantly affected days to flowering of lentil, while the main effect of sulphur and their interaction were significantly ($p < 0.05$) influenced days to flowering (Table 2). The longest days to flowering (47 and 46 days) were observed at 40 kg S ha^{-1} under no-inoculation and at 20 kg S ha^{-1}under inoculation, respectively. The shortest (45 and 45 days) were obtained under inoculated and un-inoculated seeds without S fertilization (Table 2). Every increase in S rate resulted in extending days to flowering under un-inoculated treatments, while the effect of S was peaked at 20 kg S ha^{-1} under inoculation. This might be due to the fact that applied S fertilizer increasing the adsorption sites which plays an important role in vegetative growth of plants as it is a component of ferredoxin in chloroplast and involved in photosynthetic processes [22]. *Rhizobium* inoculants were also enhanced N fixation and improve S uptake in the soil, which may contribute to improved vegetative growth of lentil at optimum S rates. Similarly, application of 15 kg S ha^{-1}under inoculation on chickpea take more days to reach 50% flowering than 30 kg S ha^{-1} under inoculation [23]. On the other hand, days to flowering increased with increasing sulphur levels up to 40 kg ha^{-1} [24].

3.2. Number of Nodules Plant^{-1}

Number of nodules $plant^{-1}$ (NNP) were not significantly influenced by the main effect of *Rhizobium* inoculants and sulphur application, while the interaction of *Rhizobium* inoculants and sulphur was significantly ($p < 0.05$) influenced NNP (Table 2). The highest NNP (16) was obtained at the rate of 20 kg S ha^{-1} along with seed inoculated by *Rhizobium*, while the lowest (12) was recorded at 40 kg S ha^{-1} under inoculation (Table 2). However, NNP was statistically similar between S rates of 20 and 40 kg ha^{-1}without inoculation. Under inoculated treatments, the NNP between control and 20 kg S ha^{-1} application did not vary significantly, while further increase to 40 kg S ha^{-1} depressed NNP. The result indicated that S application has shown a tendency to be relatively more important in influencing NNP when seeds are not inoculated. The positive effect of *Rhizobium* inoculation and S may have created favorable soil condition for the growth and development of nitrogen fixing bacteria and promote the utilization of high quantities of nutrients through their well-developed root system for better nodules [25]. Sulphur fertilization and inoculation also significantly increased NNP, fresh weight and volume of nodules with optimum rate of S in Faba bean [26].

3.3. Nodules Dry Weight Plant^{-1}

The main effect of *Rhizobium* inoculant was not significantly affected nodules dry weight $plant^{-1}$ (NDWP), while the main effect of sulphur and their interaction were significantly ($p < 0.05$) influenced NDWP (Table 2). The application of 40 kg S ha^{-1} under *Rhizobium* inoculation gave the highest mean NDWP (1.1mg) followed by no inoculation with the same S rate, while the lowest (0.7 mg) was recorded at control treatment (Table 2). As increasing S level with and without *Rhizobium* inoculation, the mean value of NDWP increased significantly. However, NDWP was statistically par at the rate of 20 kg S ha^{-1} with and without *Rhizobium* inoculation. This positive role of S and *Rhizobium* inoculants in NDWP might be due to the fact that, both nutrient plays a vital role in plant metabolism and positively affects the nodulation in plants. Consistent with this finding, root and nodule development of legumes root is promoted by S fertilization [27]. Similarly, dry weight of nodules increased at combined application S and *Rhizobium* inoculation in soybean plants [28].

Table 2. *Interaction effects of Rhizobium inoculant and sulphur application on phenological and nodule parameters of lentil.*

Treatments	Days to flowering (Days)		Number of nodules $plant^{-1}$		Nodules dry weight $plant^{-1}$ (mg)	
	Rhizobium Inoculant					
	Inoculated	un-inoculated	Inoculated	un-inoculated	Inoculated	un-inoculated
Sulphur ($kgha^{-1}$)						
0	44.8^{d}	44.6^{d}	14.1^{ab}	12.8^{b}	0.8^{d}	0.7^{e}
20	46.1^{ab}	45.4^{c}	15.6^{a}	13.6^{ab}	0.9^{c}	0.9^{c}
40	45.5^{bc}	46.5^{a}	12.4^{b}	14.3^{ab}	1.1^{a}	1.0^{b}
LSD (0.05)	0.59		2.10		0.06	
CV (%)	1.57		18.6		7.8	

Means with the same letter in the columns and rows are not significantly different at 5% probability level. Where LSD = least significant difference, CV = coefficient of variation.

3.4. Number of Seeds Pod^{-1}

The main effects of *Rhizobium* inoculant as well as interaction effects of sulphur and *Rhizobium* inoculant were significantly (P < 0.01) influenced number of seeds pod^{-1}. However, main effect of S was non-significant (Table 3). Application of 20 kg S ha^{-1}under inoculation gave the highest number of seeds pod^{-1} (2), while the lowest (1) was obtained from the control treatment (Table 3). The application of S at 20 kg ha^{-1} only resulted in significantly increased number of seeds pod^{-1} over the other treatments under inoculation. However, without inoculation, any levels of S did not exhibit significant impact on number of seeds pod^{-1}. This could be due to the fact that *Rhizobium* inoculation adequately supply N for the plant and resulted in increased chlorophyll synthesis and photosynthetic products. Sulphur also increase leaf size and photosynthetic materials and resulted increase number of seeds pod^{-1} [29].

3.5. Total Aboveground Dry Biomass

The main effects of S and *Rhizobium* inoculant as well as their interactions of S with *Rhizobium* inoculant were significantly (p < 0.01) influenced total aboveground dry biomass (Table 3). The highest total aboveground dry biomass (8.5 t ha^{-1}) was obtained under seed inoculated by *Rhizobium* without S fertilization, while the lowest (6.3 t ha^{-1}) was recorded at 20 kg S ha^{-1} under un-inoculation (Table 3). Even thought, the highest total aboveground dry biomass was obtained at nil application of S rate with inoculation, application of 20 kg S ha^{-1} under inoculation was statistically similar. Without seed inoculation, application of sulphur at 20 and 40 kg ha^{-1} resulted in significantly lower total aboveground dry biomass of lentil in comparison with the control treatment. The increase in total aboveground dry biomass of lentil in response to *Rhizobium* inoculation could be due to sufficient nitrogen supply mainly from BNF and sulphur also necessary for enzymatic action, chlorophyll formation, synthesis of certain amino acids and vitamins. Nitrogen fixation by *Rhizobium* hastened the vegetative growth of legume crops and S application improve the soil condition and nutrient uptake, which are the possible reasons for substantial increase of biological yield [30].

3.6. Seed Yield

Seed yield of lentil was significantly (P < 0.05) affected by the main effects of sulphur and *Rhizobium* inoculation. Similarly, interaction effects of sulphur with *Rhizobium* inoculation showed highly significant (p < 0.01) effects on seed yield of lentil (Table 3). The highest seed yield (2.3 t ha^{-1}) was observed at 40 kg S ha^{-1} under no-inoculation and seed inoculation without S fertilization. However, this treatment was statistically at par to all other combinations except the control treatment that produced the lowest (1.9 t ha^{-1}) yield (Table 3). The higher yield due to S fertilization might be due to increased photosynthesis on one hand and greater mobilization of photosyntyhates towards reproductive structures, on the other, leading to a significant increase in yield attributes of lentil. The conflicting effect of *Rhizobium* inoculants with S on seed yield may be attributed to the tendency of higher rates of nitrogen through BNF, enhance vegetative growth that might have resulted in self-shading thereby reducing the overall yield. Application of S with Rhizobium inoculation also significantly increased seed and straw yield in mungbean [31].

Table 3. *Interaction effects of Rhizobium inoculant and sulphur application on yield and yield components of lentil.*

Treatments	Number of seeds pod^{-1}		Total aboveground dry biomass (t ha^{-1})		Seed yield (t ha^{-1})	
	Rhizobium Inoculant					
	Inoculated	un-inoculated	Inoculated	un-inoculated	Inoculated	un-inoculated
Sulphur (kg ha^{-1})						
0	1.58^{b}	1.39^{b}	8.47^{a}	7.97^{ab}	2.25^{a}	1.94^{b}
20	1.99^{a}	1.54^{b}	8.22^{a}	6.31^{d}	2.09^{ab}	2.14^{ab}
40	1.61^{b}	1.57^{b}	7.45^{bc}	7.25c	2.12^{ab}	2.27^{a}
LSD (0.05)	0.23		0.71		0.22	
CV (%)	17.29		11.41		12.41	

Means with the same letter in the columns and rows are not significantly different at 5% probability level. Where LSD = least significant difference, CV = coefficient of variation.

3.7. Sulphur Uptake

Seed, haulm and total sulphur uptake were significantly (p < 0.001) influenced by the main effect of sulphur and their interaction of *Rhizobium* inoculant and sulphur application. Moreover, main effect of *Rhizobium* inoculant showed a significant (p < 0.05) effect on seed and total S uptake and non-significant on haulm S uptake (Table 4). Maximum seed sulphur uptake (4 kg ha^{-1}) was recorded under inoculation without S fertilization, which was significantly higher than the other treatments (Table 4). Thus, seed S uptake that resulted from inoculation without S fertilization exceeded the seed S uptake obtained in the control by about 79%. With respect to un-inoculated, seed S uptake significantly improved as S rate successively increased. However, seed S uptake was decreased significantly with increasing levels of sulphur from nil to 40 kg ha^{-1}. The higher seed S uptake due to inoculation could be attributed to the fact that some isolates of Rhizobia have the ability increased availability of sulphur in the soil and thereby increase seed S uptake in plants.

The highest haulm sulphur uptakes (23 and 21 kg ha^{-1}) were observed without S fertilization under inoculation and at 40 kg S ha^{-1} under no inoculation, respectively. Whereas, the minimum (12 kg ha^{-1}) was obtained from both inoculated at 40 kg S ha^{-1} and un-inoculated plants without S fertilization (Table 4). Every increase in S rate resulted in significantly decreased haulm sulphur uptake under inoculated treatments, while the effect of S under no inoculation the reverse is true. The increase in haulm sulphur uptakes with increased sulphur levels under no inoculation could be due to increased availability of sulphur in the soil as a result of the applied fertilizer. An increase in HSU due to inoculation with lower S rate could be related to a positive effect of *Rhizobium* inoculation in nutrient uptake. The interaction of S and *Rhizobium* inoculation is synergistic at optimum rates and antagonistic at excessive levels of one of them [32]. Integrated application of *Rhizobium* strains and S could be a viable strategy to improve the S uptake in seed and haulm on soybean [33]. They concluded that at higher rate of S with *Rhizobium* the S uptake in seed and haulm declined due to unbalanced S application in soil.

Maximum total S uptake (25 kg ha^{-1}) was recorded at the combined application of *Rhizobium* inoculants without S fertilization, while the minimum total S uptake (13 kg ha^{-1}) was obtained from the control (Table 4). Seed inoculation with sulphur from nil to 40 kg ha^{-1} significantly decreased total S uptake. However, under no inoculation with sulphur from nil to 40 kg ha^{-1}total S uptake also significantly influenced with increasing mean value. Generally, an increase in total S uptake due to inoculation and S could be related to the significant increase in seed and haulm S uptake resulting in higher accumulation of total S uptake in plants. However, haulm S uptake more contributed to total S uptake than seed S uptake.

Table 4. *Interaction effects of Rhizobium inoculant and sulphur application on sulphur uptake.*

Treatments	Seed S uptake (kg ha^{-1})		haulm S uptake (kg ha-1)		Total S uptake (kg ha-1)	
	***Rhizobium* Inoculant**					
	Inoculated	**un-inoculated**	**Inoculated**	**un-inoculated**	**Inoculated**	**un-inoculated**
Sulphur (kg ha^{-1})						
0	3.88^{a}	1.03^{d}	23.33^{a}	11.88^{c}	24.89^{a}	12.91^{c}
20	1.68^{c}	1.56^{c}	15.24^{b}	13.53^{bc}	16.51^{b}	15.07^{b}
40	1.27^{d}	2.04^{b}	11.82^{c}	21.31^{a}	13.50^{c}	23.29^{a}
LSD (0.05)	0.26		2.08		2.04	
CV (%)	16.64		15.65		13.74	

Means with the same letter in the columns and rows are not significantly different at 5% probability level. Where LSD = least significant difference, CV = coefficient of variation.

3.8. Sulphur Use Efficiency and Harvesting Index

Sulphur agronomic efficiency, recovery efficiency and harvesting index of lentil were significantly ($p < 0.001$) influenced by the main effect of sulphur and their interaction of *Rhizobium* inoculant and sulphur application. However, main effect of *Rhizobium* inoculant was not significant (Table 5). Maximum sulphur agronomic efficiency (32 kg kg^{-1}) was obtained from the combined application of 20 kg S ha^{-1} with *Rhizobium* inoculant, while the minimum value of (15kg kg^{-1}) was recorded at the combined application of 20 kg S ha^{-1} without *Rhizobium* inoculation (Table 5). The result also showed as increasing S rates from 20 to 40 kg ha^{-1} with and without inoculation the values of sulphur agronomic efficiency was significantly decreased. However mean value of sulphur agronomic efficiency under inoculation greater than un- inoculated at each rate. This might be due to a positive effect of *Rhizobium* inoculants and small amounts of applied fertilizer optimized nutrient use efficiency [34].

The maximum sulphur recovery efficiency (66%) and minimum (25%) were recorded at the rate of 20 and 40 kg S ha^{-1} along with *Rhizobium* inoculants, respectively (Table 5). Every increasing S rate with and without inoculation sulphur recovery efficiency decreased. In addition, higher recovery is indicative of a more efficient uptake while higher yield is necessary for a more efficient utilization of the sulphur taken up by the plants. It is interesting to note that S uptake in seed, straw and total S in plant declined as S levels increased with *Rhizobium* inoculation. Increase in sulphur recovery efficiency due to sulphur application and seed inoculation have also been reported by the study [35].

Significantly highest sulphur harvest index (18%) was recorded under *Rhizobium* inoculants without S fertilization followed by application of 40 kg S ha^{-1} without *Rhizobium* inoculation (Table 5). The lowest sulphur harvest index (8%) was also obtained at the rate of 40 kg S ha^{-1} under inoculation. Under inoculated treatments, sulphur harvest index was significantly varied as S rate increase, but without inoculation sulphur harvest index was not varied each other. Generally, the result showed that S application has shown a tendency to be more important in influencing sulphur harvest index when seeds are not inoculated.

Table 5. *Interaction effects of Rhizobium inoculant and sulphur application on sulphur use efficiency and harvest index.*

Treatments	Sulphur agronomic efficiency (kg ka^{-1})		sulphur recovery efficiency (%)		sulphur harvest index (%)	
	Inoculant		**Inoculant**		**Inoculant**	
	Inoculated	**un-inoculated**	**Inoculated**	**un-inoculated**	**Inoculated**	**un-inoculated**
Sulphur (kg ha^{-1})						
0	---	---	---	---	---	---
20	32.11^{a}	15.54^{d}	66.00^{a}	61.65^{ab}	11.19^{c}	12.96^{bc}

Treatments	Sulphur agronomic efficiency (kg ka^{-1})		sulphur recovery efficiency (%)		sulphur harvest index (%)	
	Inoculant		Inoculant		Inoculant	
	Inoculated	un-inoculated	Inoculated	un-inoculated	Inoculated	un-inoculated
40	29.35^{b}	19.34^{c}	25.20^{c}	54.83^{b}	17.68^{a}	15.54^{ab}
LSD (0.05)	2.62		8.02		3.07	
CV (%)	19.59		18.50		28.57	

Means with the same letter in the columns and rows are not significantly different at 5% probability level. Where LSD = least significant difference, CV = coefficient of variation.

3.9. Partial Budget Analysis

According to the results of partial budget analysis, the highest net benefit were obtained from the application of *Rhizobium* inoculation without S fertilization (ETB 53258 ha^{-1}) followed by 20 kg S ha^{-1} without *Rhizobium* inoculation (ETB 51628 ha^{-1}) and 40 kg S ha^{-1} with *Rhizobium* inoculation (ETB 44850 ha^{-1}) (Table 6). According to dominance analysis, as indicated on (Table 6) most of the treatments were dominated by the highest net benefit treatments hence, eliminated for further economic analysis.

To identify treatments with maximum return to the farmers' investment, marginal analysis was performed on non-dominated treatments. For a treatment to be considered as a worthwhile option to farmers, the marginal rates of return (MRR) need to be at least between 50% and 100% [21]. Thus, to draw farmer' recommendations from marginal analysis in this study, 100% return to the investment is a reasonable minimum acceptable rate of return since farmers' in the study area usually not apply combined application of S and *Rhizobium* inoculation for lentil production. Accordingly, *Rhizobium* inoculation without S (10271% ^{MRR}) was superior rewarding treatment combination. This implies that for Birr 1.0 investment in lentil production, the producer can get Birr 102. Therefore, farmers in Denkaka and similar agro-ecology condition use *Rhizobium* inoculants to produce higher yield. From this finding it was observed *Rhizobium* inoculation was crucial for lentil production.

Table 6. *Partial budget analysis.*

Treatment		Adjusted Yield. (kg ha^{-1})	Adjusted. Straw (kg ha^{-1})	Total variable Cost (ET birr)	Net-benefit	MRR%
Skg ha^{-1}	Rhkg ha^{-1}					
0	0	1274	4109	0	36825 (D)	
0	0.21	1969	4925	160	53258	10271
20	0	2034	4861	2986	51628 (D)	
20	0.21	1433	5367	3146	40093 (D)	
40	0	1984	3022	5972	43095 (D)	
40	0.21	1887	4630	6132	44830 (D)	

Where: S = sulphur, Rh = *Rhizobium* inoculants, D = dominated, MRR = marginal rate of return and ET birr = Ethiopian birr.

4. Conclusion

The field experiment indicated that S fertilization improved all variables studied except number of nodule $plant^{-1}$ and number of seed pod^{-1}, consequently the improvements were more pronounced for the combined application of sulphur under inoculation than their separate application. *Rhizobium* inoculation also significantly improved certain yield and yield components as well as sulphur uptake of lentil. However, days to flowering, nodulation parameters such as number of nodule $plant^{-1}$ and nodule dry weight and nutrient use efficiency such as agronomic efficiency, recovery efficiency and harvest index of lentil were not significantly influenced by the main effect of *Rhizobium* inoculant. Joint application of *Rhizobium* inoculant and sulphur resulted in maximum values of days to flowering, number of nodules $plant^{-1}$, nodule dry weight $plant^{-1}$, number of seed pod^{-1}, aboveground dry biomass, seed yield and sulphur uptake as well as nutrient use efficiency parameters followed by the individual treatments of sulphur and *Rhizobium* inoculant. This enhancement may be due to, addition of sulphur and *Rhizobium* inoculant to soil causes a series of chemical transformations leads to accumulation of organic matter that alleviates soil characters favor *Rhizobium* growth and activity, which in turn, owe to optimal nitrogen fixation and production of huge beneficial compounds which reflected positively on lentil crop. Application of the dual treatment of 40 kg S ha^{-1}without *Rhizobium* inoculant and *Rhizobium* inoculant with nil application of sulphur were effective strategy for improving seed yield and sulphur uptake of lentil. The best recommendation for treatments based on high net benefit, relatively low variable cost together with an acceptable and maximum MRR becomes the tentative recommendation. Therefore, it can be recommended that *Rhizobium* inoculation without S application was the best treatment for farmers due to acceptable and highest MRR. However, it is difficult to make a definite and draw sound recommendation based on one location and one season experiment. So, attention shall be given to conducting similar research over locations and seasons would be relevant to get conclusive result and the effectiveness of these commercial inoculants of lentil with respect to soil fertility status and cropping system need further investigation.

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