



Effect of Nitrogen and Sulphur on Nitrogen and Sulphur Uptake in Linseed (*Linum usitatissimum* L.) in Sinana, Southeastern Ethiopia

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To cite this article:

Reta Dargie Gudeta, Nigussie Dechassa. Effect of Nitrogen and Sulphur on Nitrogen and Sulphur Uptake in Linseed (*Linum usitatissimum* L.) in Sinana, Southeastern Ethiopia. *International Journal of Applied Agricultural Sciences*. Vol. 3, No. 5, 2017, pp. 111-116.

doi: 10.11648/j.ijaas.20170305.12

Received: July 7, 2017; **Accepted:** July 25, 2017; **Published:** August 16, 2017

Abstract: Linseed (*Linum usitatissimum* L.) has been a traditional crop and it is the most important oil seed crop in production in the higher altitudes of Ethiopia. The needs for applying fertilizers are becoming obvious, as soil fertility has declined from time to time. Excessive use of fertilizers also affects farmers' economy, as the crop is relatively low yielder. In order to study the effect of nitrogen and sulphur on nitrogen and sulphur uptake of the crop, field experiment was conducted in factorial based randomized complete block design with three replications at Sinana during the main cropping season of 2013. Treatments consist of four levels of nitrogen (0, 23, 46 and 69 kg N ha⁻¹) and four levels of sulphur (0, 20, 40, 60 kg S ha⁻¹). The results indicated that increased nitrogen application combined with sulphur increased seed nitrogen uptake as compared with the control plot (no nitrogen and sulphur). However, increasing sulphur application combined with nitrogen at 69 kg ha⁻¹ progressively increased seed nitrogen uptake, but it showed a declining trend combined with the rest of nitrogen levels. Seed sulphur uptake was enhanced by the application of sulphur and it was higher at higher levels of both sulphur and nitrogen. However, to come up with a conclusive recommendation, the experiment should be repeated over seasons and locations.

Keywords: Linseed, Nitrogen Uptake, Sulphur Uptake

1. Introduction

Linseed (*Linum usitatissimum* L.) has been a traditional crop in Ethiopia and it is the second most important oil seed crop in production after noug (*Guizotia abyssinica* Cass) in the higher altitudes (Adugna and Adefris, 1995) and both crops contribute over 75% to the total annual production. Ethiopia is considered the secondary center of diversity, and now the 5th major producer of linseed in the world after Canada, China, United States and India (Adugna, 2007).

Linseed oil extracted from the seed is used for human consumption and contains α -linolenic acid (ALA), a polyunsaturated fatty acid that has nutritional and health benefits. The oil is also used as a raw material for a number of industrial products, such as drying agents, paintings and varnishes, soap manufacture, printing inks etc., whereby it

lends itself as an export commodity, thus, contributing a lot towards building the national economy. The pressed cake, after the extraction of oil is a good protein rich livestock feed. The fibre type linseed is used for making high quality clothes. Linseed is also suitable in crop rotation programs with cereals.

The crop performs best in altitudes ranging from 2200 to 2800 meters above sea level (masl). Linseed is suited to a wide range of soil types but establishment can be difficult on heavy clay soils, as soils of a gravelly or dry sandy nature, which has to be avoided. A fine tilth and adequate moisture are required to ensure good establishment. Linseed requires cool temperature during its growing period for better yield.

Fimes *et al.*, 2000 reported that the synergistic interaction between N and S often lead to higher crop yield, better crop quality in terms of protein, S containing amino acids, oil and

enhances NUE and SUE. Increasing linseed yield as well as oil yield requires optimum balanced nutrition including sulphur, which is a key nutrient for linseed oil quality (Franzen, 2004). The combined effect of N and S are better than the N and S applied alone, because they are highly inter-related in the synthesis of proteins (McGrath and Zhao, 1996). The severity of S deficiency is aggravated by higher rates of N application. Plants grown without N fertilizer showed no apparent S stress, whereas plant receiving N fertilizer particularly at higher rate without S, showed symptoms suggesting severe physiological disorder in N nutrition (Kopriva and Rennenberg, 2004).

The Bale highlands are one of the potential areas for the production of highland oil crops in Ethiopia, and linseed is the main oil crop grown in the zone. Despite its potential and profitability, the crop has been traditionally grown on marginal lands with or without fertilizers (Adefris *et al*, 1992). Hence, it is important to incorporate improved management practices to the ever-increasing need of farmers to this crop for higher seed yield. Keeping in view the importance of Nitrogen and Sulphur, the field experiment was conducted to see the effect of N and S on content and uptake of nitrogen and sulphur in seed and straw of linseed in Sinana, southeastern Ethiopia.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was conducted on research field of Sinana Agricultural Research Center in the highlands of Bale, Southeastern Ethiopia during the main cropping season of 2013. Sinana is located at a distance of 463 km from Addis Ababa at about 7°07'N latitude, 40°10'E longitude, and at an altitude of about 2400 meters above sea level. The area is characterized by a bimodal rainfall pattern. There are two growing seasons locally called 'Bona' and 'Gana' based on the time of crop harvest. *Bona* extends from August to December and *Gana* from March to July. The area receives rainfall of 346 to 861 mm during the first rainy season (March to July) and 353 to 894 mm during the main season (August to December). The mean annual maximum temperature is 21°C and the monthly values range between 19.4°C in October and 22.8°C in February. The mean annual minimum temperature is 9.5°C and the monthly values range between 7.7°C in December and 10.7°C in April. The coldest month is December whereas February is the hottest month.

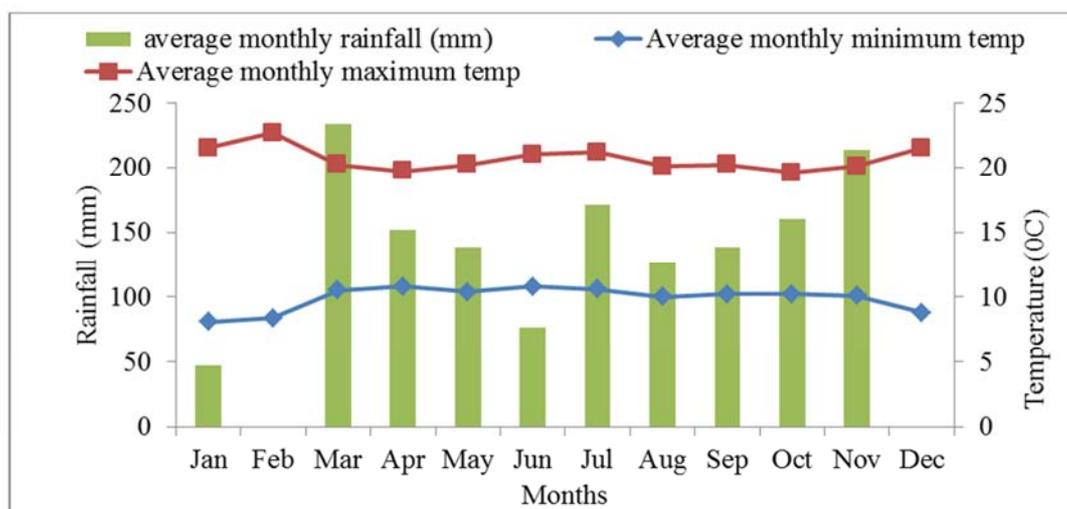


Figure 1. Average monthly rainfall (mm) and temperature (°C) in the experimental year.

2.2. Treatments and Experimental Design

The linseed variety used for the study was *Jiituu* (CI-1652 x Omega/B/53), which was released by Sinana Agricultural Research Centre in 2012. Urea was used as a source of N fertilizer and gypsum as S fertilizer. A uniform rate of 23 kg P₂O₅ha⁻¹ was also applied uniformly for all plots as a basal. The treatments consisted of four rates of nitrogen (0, 23, 46, and 69 kg N ha⁻¹) and four rates of sulphur (0, 20, 40, and 60 kg S ha⁻¹). The experiment was laid out in a randomized complete block design (RCBD) in a factorial arrangement replicated three times. The field layout was prepared and the treatments were assigned to each experimental plot randomly within a block. Each plot consisted of 4 meter long 10 rows spaced 20cm apart. The size of each plot was 4.0m x 2.0m

(8m²). Adjacent replications, blocks, and plots were separated by a 1.5m, 1m, and 1m, distances, respectively. The net central unit area of each plot consisted of 6 rows for sample measurements, leaving aside plants in the four outer rows and those at both ends of each of the rows to avoid border effects.

2.3. Experimental Procedures

2.3.1. Field Activities and Treatment Application

Linseed seed was sown in to rows at the recommended rate of 25 kg ha⁻¹ on the prepared fine seedbed on August 09, 2013. Nitrogen fertilizer in the form of urea was applied at the specified rates in two equal parts, *i.e.*, half was applied at sowing and the remaining half was top-dressed just at the

start of flowering stage. Sulphur was applied in the form of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) at the specified rates at sowing. The full dose of P was applied uniformly to all plots at the recommended rate of $23 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ at sowing. All broadleaved and grass weeds were removed by hand weeding 30-35 days after emergence. Harvesting was performed at maturity on 21 January 2014.

2.3.2. Soil Sampling, Preparation and Analysis

One composite soil sample per replication, each made from five sub-samples, was collected from the depth of 0-30 cm before planting. The samples were air-dried, ground using a pestle and a mortar and allowed to pass through a 2 mm sieve. The samples were analyzed for selected physico-chemical properties mainly organic carbon, total nitrogen, available sulphur, available P, exchangeable bases (Na, K, Ca and Mg), cation exchange capacity (CEC), pH, bulk density and particle size distribution, using standard laboratory procedures. Similarly, surface soil samples at 0-30cm depth were collected from five spots in each treatment in a zigzag

pattern after harvesting the experimental plots. The samples from the plots with the same treatment were bulked to form 16 composite soil samples and analyzed for total N and available S.

2.3.3. Plant Tissue Sampling, Determination of Seed and Straw Nitrogen and Sulphur Content

After physiological maturity, plant samples in the net plot were harvested at ground level and separated into seed and straw. The seed and straw sample materials were separately air-dried ground and sieved through 1mm size sieve to prepare a sample of 10g for laboratory analysis of N and S concentrations. Total nitrogen in the grain and straw sub-samples were quantitatively determined by following the micro-Kjeldhal (Jackson, 1962) digestion method with sulphuric acid. Sulphur in seed and straw sub-samples was determined turbidimetrically using a spectrophotometer by di-acid ($\text{HNO}_3\text{-HClO}_4$) digestion as stated in FAO guide to laboratory establishment for plant nutrient analysis (FAO, 2008).

3. Results and Discussion

3.1. Soil Physical and Chemical Properties

Table 1. The results of soil analysis, which were done before sowing of linseed, are presented in.

Parameter	Method	Unit	Value
pH	Potentiometric	1:2.5	6.73
Na^+	Neutral Ammonium acetate-Flame photometry	$\text{cmol}(+) \text{ kg}^{-1}$	4.20
K^+	Neutral Ammonium acetate-Flame photometry	$\text{cmol}(+) \text{ kg}^{-1}$	2.82
Mg^+	Atomic Absorption Spectrophotometer	$\text{cmol}(+) \text{ kg}^{-1}$	8.1
Ca^+	Atomic Absorption Spectrophotometer	$\text{cmol}(+) \text{ kg}^{-1}$	17.8
CEC	Neutral Ammonium acetate -Ammonia distillation	$\text{cmol}(+) \text{ kg}^{-1}$	48.56
Tot. N	Kjeldahl Bremmer, J. M. and C. S. Mulvancy, 1982	%	0.17
Av. S	Monocalcium phosphate extract-Turbidimetry	mg kg^{-1}	22.17
Av. P	Olsen method	ppm	10.12
OC	Walkley, A., and Black, I. A. 1934	%	2.77
Bulk density	Core sampler method	g cm^{-3}	1.02
Texture	Hydrometer		Sand= 26
			Silt= 23
			Clay= 53
		Textural class	Clay

3.2. Total Nitrogen and Available Sulphur Contents of the Soil After Harvest

Soil total nitrogen and available sulphur contents were analyzed for composited soil samples from each treatment to assess the post harvest status of the soil. Total nitrogen values did not show wide variation due to different treatment combinations. Although soil analysis after harvest showed

inconsistent results, higher rates of sulphur application resulted in more available soil sulphur content. However, the values were lower than those recorded before planting (Table 2). This may be attributed to increased plant uptake and, thus, concentration of sulphur in the crop. The lowest mean available sulphur of the soil after harvest was observed at the highest nitrogen level, *i.e.* 69 kg N ha^{-1} . This variation could be due to better utilization of sulphur at higher nitrogen levels.

Table 2. Effect of nitrogen and sulphur on total nitrogen and available sulphur of the soil.

N levels (kg ha^{-1})	Total nitrogen (%)				Mean	Available sulphur (mg kg^{-1})				Mean
	S levels (kg ha^{-1})					S levels (kg ha^{-1})				
	0	20	40	60		0	20	40	60	
0	0.17	0.16	0.16	0.17	0.165	8.87	11.08	8.87	22.17	12.75
23	0.17	0.18	0.17	0.17	0.173	7.76	6.1	16.62	6.65	9.28
46	0.17	0.18	0.16	0.17	0.170	4.43	9.98	8.87	18.84	10.53
69	0.16	0.18	0.17	0.17	0.170	6.10	8.87	7.76	12.19	8.73
Mean	0.168	0.175	0.165	0.170		6.79	9.00	10.53	14.96	

3.3. Effect of Nitrogen and Sulphur on Nitrogen and Sulphur Content in Seed and Straw

Seed and straw nitrogen Content

Nitrogen content of the seed varied from 2.96 to 3.44% and straw nitrogen content varied from 0.26 to 0.74% (Table 3). It was observed that increased application of N levels combined with sulphur consistently increased grain N content over the control, except at 60 kg S ha⁻¹, while the straw N content generally decreased with increased N levels except for 46 kg N ha⁻¹. The increase in seed N content of linseed with the application of N and S could be due to the fact that N is an integral part of protein and the protein of linseed involves S containing amino acids like methionine and cystine. The increase in seed N content attained observed in the present study was in agreement with the findings of Dubey *et al.* (1995) and Kutcher *et al.* (2005) who reported that seed N content of canola increased significantly with increasing N rates. However, the combined effect of S and nitrogen application was inconsistent on seed and straw N contents. This might be attributed to the variability in soil S,

though the available sulphur content of the experimental site was medium.

Increased levels of sulphur combined with 23 and 46 kg N ha⁻¹ consistently increased seed sulphur content which decreased in the absence of nitrogen application. The highest seed S concentration (0.087mg kg⁻¹) was observed for the highest rate of sulphur (60 kg S ha⁻¹) at 23 kg N ha⁻¹. The effect of nitrogen on seed sulphur content of linseed was inconsistent. However, the maximum seed sulphur content was observed for 23 kg N ha⁻¹ combined with the maximum sulphur level (60 kg S ha⁻¹). Sulphur contents of grain and straw were less than the corresponding N contents. This is because the concentration of sulphur in plant tissues is much lower (about 10 times less) than that of nitrogen (Marschner, 1995). Sulphur is an essential element as a constituent of proteins, cysteine-containing peptides, such as glutathione, or numerous secondary metabolites in plants (Scherer *et al.*, 2001) and for synthesis of vitamins and Chlorophyll in the cell (Kacar and Katkat, 2007).

Table 3. Effect of nitrogen and sulphur on nitrogen and sulphur content in seed and straw.

N (kg ha ⁻¹)	Seed N content (%)					Straw N content (%)				
	S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)				
	0	20	40	60	Mean	0	20	40	60	Mean
0	3.02	3.01	3.02	3.17	3.055	0.35	0.38	0.28	0.36	0.343
23	3.04	3.15	3.12	2.96	3.068	0.26	0.34	0.38	0.35	0.333
46	3.17	3.16	3.19	3.05	3.143	0.74	0.36	0.34	0.39	0.458
69	3.44	3.42	3.37	3.15	3.345	0.4	0.29	0.34	0.33	0.340
Mean	3.168	3.185	3.175	3.083		0.438	0.343	0.335	0.358	
N (kg ha ⁻¹)	SeedS content (mg kg ⁻¹)					StrawS content (mg kg ⁻¹)				
	S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)				
	0	20	40	60	Mean	0	20	40	60	Mean
0	0.095	0.071	0.063	0.063	0.073	0.015	0.031	0.023	0.024	0.023
23	0.071	0.079	0.079	0.087	0.079	0.024	0.016	0.016	0.024	0.02
46	0.039	0.063	0.071	0.086	0.065	0.031	0.008	0.024	0.016	0.019
69	0.0979	0.079	0.069	0.071	0.075	0.008	0.016	0.024	0.024	0.018
Mean	0.0071	0.0073	0.071	0.077		0.019	0.017	0.022	0.022	

3.4. Effect of Nitrogen and Sulphur Levels on Nitrogen and Sulphur Uptake in Seed and Straw of Linseed

3.4.1. Seed and Straw Nitrogen Uptake

All plants require the same mineral elements. However, the quantity, rate and timing of uptake vary with crop, variety, climate, soil characteristics and management. These combined factors influence the nutritional need, nutrient content, and overall yield of a crop. Information on nutrient uptake of the crop is crucial in determining the fertilizer use efficiency, and also helpful in devising fertilizer management strategies for achieving yield targets in a crop. Uptake of a nutrient element by the plant is estimated as dry matter multiplied by concentration of the given nutrient. The nitrogen and sulphur uptake in the seed and straw of linseed are presented in (Table 4).

Increased nitrogen application combined with sulphur increased seed nitrogen uptake as compared with the control plot (no nitrogen and sulphur). In addition, a consistent

increment was observed at 40 and 60 kg S ha⁻¹ as combined with increasing nitrogen application. The maximum seed nitrogen uptake (73 kg ha⁻¹) was recorded at 69 kg N ha⁻¹ combined with 40 kg S ha⁻¹, which improved seed nitrogen uptake by 30.95% over plots receiving no N/S. This result confirms that the combined effect of S and N played a significant role in N uptake by linseed plants. The results were in agreement with the findings of Chaubey *et al.* (1993) who observed that increased N uptake of linseed grain and straw was obtained by the application of sulphur in sandy and loam soils.

Straw and total nitrogen uptake was consistently increased with increasing nitrogen application combined with 60 kg S ha⁻¹. Sulphur application increased seed nitrogen uptake when only combined with 69 kg ha⁻¹ nitrogen. This implies that with higher levels of S and N application, N uptake was comparatively higher (73 kg ha⁻¹). Therefore, sulphur is not only important in the growth of linseed, but also in nitrogen utilization. However, increased sulphur application combined

with higher nitrogen levels showed a declining trend. Mean straw and total nitrogen uptake generally declined with increased sulphur application.

3.4.2. Seed and Straw Sulphur Uptake

Increased nitrogen fertilizer combined with sulphur levels progressively increased the seed sulphur uptake (Table 4). Jaggi *et al.* (1995) have reported that application of S and N increased their respective uptake by linseed. This may be attributed to the favourable effect of nitrogen on growth and yield attributes of linseed, which ultimately increases seed and straw yields and consequently results in higher uptake of nitrogen and sulphur by the crop. The highest mean seed sulphur uptake (1.8 kg ha⁻¹) was observed at 40 or 60 kg S ha⁻¹ combined with 23 N kg ha⁻¹, which gave 12.90% higher seed sulphur uptake than no sulphur as combined with nitrogen.

Seed sulphur uptake was enhanced by the application of sulphur and it was higher at higher levels of both sulphur and nitrogen. This may be attributed to the higher requirement of sulphur of oilseed crop as compared with cereals and pulses, in which it plays a significant role in the quality and development of seeds (Salwa *et al.*, 2010). Upadhyay *et al.* (2012) also reported the increase in seed sulphur uptake with increasing sulphur rates in linseed. In this experiment, the

seed sulphur uptake ranged from 0.8 to 1.8 kg ha⁻¹ (Table 4).

Mean straw sulphur uptake increased with increasing nitrogen and sulphur levels except at 46 kg N ha⁻¹ and 20 kg S ha⁻¹ and hence, the maximum straw sulphur uptake (1.7 kg ha⁻¹) was recorded at the highest nitrogen level (69 kg N ha⁻¹) combined with the highest sulphur level (60 kg S ha⁻¹), which gave 22% higher value than the control treatment. The maximum straw sulphur uptake (1.7 kg ha⁻¹) was recorded at the highest sulphur level (60 kg S ha⁻¹) combined with the highest nitrogen level (60 kg S ha⁻¹), with 27% higher values than the control plot. The increase in straw sulphur uptake with increased sulphur levels could be due to increased availability of sulphur in the soil as a result of the applied fertilizer. This is in line with the results of Jaggi and Dixit (1995) who reported that increasing application of sulphur increased sulphur concentration and uptake in plant.

The medium and highest sulphur levels combined with increased nitrogen rates increased total sulphur uptake. In this study, the total sulphur uptake ranged from 2.2 to 3.9 kg ha⁻¹, in which, maximum was recorded at 69 kg N ha⁻¹ combined with 60 kg S ha⁻¹, which gave 38.46% higher total sulphur uptake as compared to plots that did not receive sulphur and nitrogen (Table 4).

Table 4. Effect of nitrogen and sulphur on seed and straw nitrogen and sulphur uptake, total nitrogen and sulphur uptake.

Seed N uptake (kg ha ⁻¹)						Straw N uptake (kg ha ⁻¹)					Total nitrogen uptake				
N (kg ha ⁻¹)	S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)				
	0	20	40	60	Mean	0	20	40	60	Mean	0	20	40	60	Mean
0	50.4	53.1	48.4	56.6	52.1	18.5	20.7	16.9	23.6	19.92	68.9	73.9	65.3	80.2	72.07
23	63.0	58.0	71.9	59.9	63.2	21	26.1	30.3	24.8	25.55	84.0	84.1	102.2	84.7	88.75
46	68.2	67.7	66.9	61.9	66.2	56	27.8	25.2	29.5	34.63	124.2	95.5	92.1	91.3	100.7
69	64.5	65.8	73	70.6	68.5	36.9	25.8	29	31.6	30.83	101.4	91.7	102	102.2	99.32
Mean	61.5	61.2	65.1	62.3		33.10	25.10	25.35	27.37		94.62	86.30	90.40	89.60	
Seed Suptake (kg ha ⁻¹)						Straw Suptake (kg ha ⁻¹)					Total sulphur uptake				
N (kg ha ⁻¹)	S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)					S levels (kg ha ⁻¹)				
	0	20	40	60	Mean	0	20	40	60	Mean	0	20	40	60	Mean
0	1.6	1.3	1.0	1.1	1.25	0.8	1.7	1.4	1.6	1.38	2.4	2.9	2.4	2.7	2.60
23	1.5	1.5	1.8	1.8	1.65	1.9	1.2	1.3	1.7	1.53	3.4	2.7	3.1	3.5	3.18
46	0.8	1.3	1.5	1.7	1.33	2.3	0.6	1.8	1.2	1.48	3.2	2.0	3.3	3.0	2.88
69	1.5	1.5	1.5	1.6	1.53	0.7	1.4	2.0	2.3	1.60	2.2	2.9	3.5	3.9	3.13
Mean	1.35	1.40	1.45	1.55		1.43	1.23	1.63	1.70		2.8	2.63	3.1	3.3	

4. Conclusions

Results of plant tissue analysis showed that increasing nitrogen application combined with sulphur increased seed nitrogen uptake. However, increasing sulphur application combined with nitrogen at 69 kg ha⁻¹ progressively increased seed nitrogen uptake, but it showed a declining trend combined with the rest of nitrogen levels. The maximum seed nitrogen uptake (73 kg ha⁻¹) was recorded for 69 kg N ha⁻¹ combined with 40 kg S ha⁻¹, which improved the value by 30.95% over the control plots. Progressively increased straw nitrogen uptake due to increased nitrogen application was observed at 60 kg S ha⁻¹.

With respect to mean seed and straw sulphur uptake, increased nitrogen fertilizer combined with sulphur levels

progressively increased the seed sulphur uptake. Regarding effect of increased sulphur levels combined at nitrogen on seed sulphur uptake was only the highest (1.65 kg ha⁻¹) at 23 kg N ha⁻¹ combined with increasing sulphur levels. In this study, the seed sulphur uptake was ranged from 0.8 to 1.8 kg ha⁻¹. Straw sulphur uptake was increased due to increased nitrogen applications combined with sulphur, except for 20 kg S ha⁻¹, which was lower than no sulphur. However, the effect of sulphur on straw sulphur uptake showed increment when combined with only 46 N kg ha⁻¹.

However, to come up with a conclusive recommendation, the experiment should be repeated over seasons and locations, as it is difficult to make widely applicable conclusion based on research results of only one site and season.

Acknowledgements

The author would like to acknowledge the Oromia Agricultural Research Institute (OARI) and Sinana Agricultural Research Centre (SARC) for granting and facilitating this research work. We also thank Holetta Agricultural Research Centre Soil and Plant Analysis Laboratory for their assistance in handling the analysis of soil and plant samples.

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