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RESEARCH ARTICLE

Re-evaluating the effect of sulphur fertilization on yield and nutrient uptake in a groundnut-blackgram cropping sequence

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Abstract

Comprehensive information on the direct and residual effects of elemental sulphur fertilization on yield and nutrient uptake in sequential legume-based cropping systems, particularly the groundnut–blackgram sequence, remains limited, despite growing evidence of widespread sulphur deficiency and its critical role in crop productivity. The present study was conducted to evaluate the direct and residual effects of sulphur on yield and nutrient uptake in a groundnut-blackgram cropping sequence through a pot culture experiment at the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University (TNAU), Coimbatore, in 2021. The experiment was laid out in a completely randomized design (CRD) with six levels of sulphur and three sulphur-oxidizing bacteria (SOB) treatments, each replicated four times. Elemental sulphur was applied at 0, 20, 30, 40, 50 and 60 kg S ha^{-1} and SOB was applied at three levels-no application, seed treatment and soil application. The results revealed that the application of 60 kg ha^{-1} sulphur as elemental sulphur combined with soil application of SOB at 2 kg ha^{-1} significantly enhanced seed yield, nutrient content and nutrient uptake in both crops for direct and residual effects and was on par with S at 40 kg ha^{-1}. The experiment investigated an alternative source of sulphur nutrition that could reduce sole reliance on gypsum as a sulphur source. A residual notable impact was observed, suggesting that additional sulphur application may be unnecessary for the succeeding crop.

Keywords: direct and residual effect; groundnut - blackgram; sulphur; yield and uptake

Introduction

Sulphur, a secondary macronutrient required by plants in quantities similar to phosphorus, is now considered the fourth major plant nutrient after nitrogen, phosphorus and potassium. Although its importance in agriculture has been recognized for over a century, it was long overlooked as a macronutrient because soils were believed to receive sufficient sulphur from fertilizers and atmospheric deposition. Consequently, research on sulphur nutrition in crops remained limited and available information was scarce. Recent research has revealed that the majority of arable soils across the globe have experienced sulphur deficiency for more than thirty years, although its negative impact on crop production has only recently become evident (1). The continuous use of high-analysis fertilizers containing little or no sulphur, intensive cultivation, adoption of high-yielding crop varieties and limited application of organic manures have contributed to declining soil sulphur levels. Additionally, reduced use of sulphur-based pesticides, greater sulphur removal through high crop yields and the decline in sulphur dioxide (SO_2) emissions from fossil fuel combustion have further exacerbated sulphur deficiency in soils (2). Sulphur deficiency adversely affects photosynthesis, plant growth and seed yield in both legumes and non-legumes. Sulphur is also critical for oilseed crop production.

In nodulated legumes, sulphur application supports the synthesis of sulphur-containing amino acids, enhances nitrogen content in leaves and stems and increases the amount of biologically fixed nitrogen in the soil (2). Beyond its role as an essential plant nutrient, sulphur also functions as an effective fungicide and acaricide, contributing to sulphur-induced resistance (SIR) in plants. Sulphur serves as an essential macronutrient for plants, particularly oilseeds and legumes, as it plays a key role in metabolic processes such as protein synthesis and chlorophyll formation. It is also an essential component of specific amino acids and vitamins.

Soils lacking sufficient sulphur are unable to supply the nutrient at levels required to meet crop demand, leading to sulphur-deficient plants and reduced yields. Sulphur deficiency is prevalent in soils with low organic matter content due to their high leaching potential (3). Reports indicate that leaching losses vary depending on the source of sulphur fertilizer, ranging from 72 % for ammonium Sulphate to 7 % for clay-bound sulphur (bentonite + elemental S).

Sulphur deficiency is now increasingly detected in soils previously considered adequate in sulphur worldwide. One major factor contributing to reduced crop productivity is the limited application of sulphur fertilizers. In addition to groundnut, blackgram also shows a positive response to sulphur application (4). Therefore, the present investigation was undertaken to assess the direct and residual effects of sulphur on the yield and nutrient uptake of the groundnut–blackgram cropping system, using elemental sulphur as the sulphur source.

Materials and Methods

A pot culture experiment was conducted during 2021-2022 in the Radioisotope Laboratory of the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University (TNAU), Coimbatore, using groundnut CO 7 and blackgram VBN 8 as the direct and residual test crops, respectively. The experiment was conducted in a completely randomized design (CRD) with six levels of sulphur (0, 20, 30, 40, 50 and 60 kg S ha^{-1}) and three levels of sulphur-oxidizing bacteria (SOB) *Thiobacillus thiooxydans* 1x10^{8}CFU/g) comprising no application, seed treatment (1 kg ha^{-1}) and soil application (2 kg ha^{-1} mixed with farmyard manure (FYM) with four replications.

Groundnut, the main crop, was raised from February to May 2021, followed by the residual crop, blackgram, grown from June to September 2021. The experimental soil was sandy loam in texture with a pH of 7.2 and an electrical conductivity of 0.6 dS m^{-1}. It had low organic carbon content (3.8 g kg^{-1}), low nitrogen (186 kg ha^{-1}), medium phosphorus (12.2 kg ha^{-1}), high potassium (685 kg ha^{-1}) and low sulphur concentration (7.3 ppm). The soil contained 4.5 % calcium carbonate, indicating slight calcareousness.

For groundnut, elemental sulphur in the form of bentonite sulphur, as per the treatments, along with SOB, was incorporated three weeks before sowing. In addition, the recommended dose of fertilizer (N:P:K = 17:34:54 kg ha^{-1}) was applied as a basal dose. Vermicompost at 5 t ha^{-1} was uniformly applied to all treatments in groundnut as part of the integrated nutrient management (INM) package. For blackgram, only the recommended dose of fertilizer was applied to assess the residual effect of sulphur. Nutrient uptake was calculated by multiplying the seed yield by its nutrient content (5). Available soil sulphur was estimated using the turbidimetric method (6).

Results and Discussion

Response of groundnut (direct crop) to the application of sulphur and SOB

Results revealed that sulphur and SOB application had a significant effect on the pod yield of groundnut (Table 1). The mean pod yield ranged from 14.5 g/pot in the control to 24.6 g/pot at 60 kg S ha^{-1} combined with soil application of SOB (Table 1). However, the yield recorded at 60 kg S ha^{-1} with soil-applied SOB did not differ significantly from the yields obtained with 30, 40 and 50 kg S ha^{-1} under the same SOB treatment. A similar trend was also noticed in kernel yield, which ranged from 9.4 g/pot in control to 17.6 g/pot at highest dose of sulphur (60 kg ha^{-1}) with the SOB soil application and this value remained comparable to the yields obtained at 30, 40 and 50 kg S ha^{-1} with SOB. Similar findings have been reported in earlier studies, which also observed increased groundnut yield with the application of sulphur along with vermicompost (7, 8).

Effect of sulphur and SOB application on nutrient uptake and oil content in groundnut

Nitrogen and sulphur uptake in groundnut kernels increased consistently with the application of sulphur and SOB (Table 2). Across sulphur levels, soil application of SOB recorded the highest N uptake, followed by seed treatment with SOB, while the lowest uptake was observed in the control without SOB. Among the sulphur levels, the maximum N uptake (597 mg pot^{-1}) was recorded at 60 kg S

Table 1. Effect of sulphur levels on pod and kernel yield of groundnut CO 7

S levels / SOB	Pod yield g/pot				Kernel yield g/pot			
	S1 No SOB	**S2 Seed treatment with SOB**	**S3 Soil application of SOB**	**Mean**	**S1 No SOB**	**S2 Seed treatment with SOB**	**S3 Soil application of SOB**	**Mean**
E1-0	14.5	15.2	15.4	15.0	9.4	9.9	10.1	9.8
E2-20	19.0	19.9	20.7	19.9	12.6	13.3	14.0	13.3
E3-30	19.8	21.2	22.5	21.2	13.4	14.5	15.7	14.5
E4-40	22.3	22.6	23.0	22.6	15.5	15.7	16.3	15.8
E5-50	22.5	23.4	23.8	23.3	15.9	16.5	17.0	16.5
E6-60	23.5	23.7	24.6	23.9	16.3	16.8	17.6	16.9
Mean	19.6	20.3	21.1		13.3	13.9	14.6	
	SEd		**CD (p = 0.05)**		**SEd**		**CD (p = 0.05)**	
E	0.85		1.80		0.72		1.45	
S	0.48		0.97		0.41		0.83	
ExS	1.20		2.51		1.10		2.31	

Table 2. Effect of sulphur levels on N and S uptake in the kernel of groundnut CO 7

S levels \ SOB	N uptake in kernel (mg/pot)				S uptake in kernel (mg/pot)			
	S1 No SOB	**S2 Seed treatment with SOB**	**S3 Soil application of SOB**	**Mean**	**S1 No SOB**	**S2 Seed treatment with SOB**	**S3 Soil application of SOB**	**Mean**
E1-0	308	326	334	322	16.9	20.8	24.2	20.6
E2-20	415	442	468	442	26.5	31.9	36.4	31.9
E3-30	444	483	526	484	29.5	37.7	42.4	36.3
E4-40	516	526	549	530	35.7	42.4	45.6	41.2
E5-50	521	554	575	550	37.4	46.2	49.3	44.2
E6-60	544	566	597	570	42.4	48.7	52.8	47.3
Mean	442	467	491		29.3	36.3	39.5	
		SEd	**CD (p = 0.05)**			**SEd**	**CD (p = 0.05)**	
E		27	56			4.3	8.7	
S		17	31			2.5	5.2	
ExS		34	69			9.3	18.8	

ha^{-1} with soil application of SOB, whereas the lowest uptake (308 mg pot^{-1}) occurred in the absolute control without SOB. The mean N uptake increased from 322 mg pot^{-1} at 0 kg/ha sulphur to 570 mg pot^{-1} at 60 kg/ha sulphur, indicating a strong positive response to increased sulphur supply. Both the main effects of sulphur and SOB, as well as their interaction were statistically significant.

A similar trend was observed for sulphur uptake in kernels. The highest S uptake (52.8 mg pot^{-1}) was recorded under 60 kg S ha^{-1} with soil application of SOB, whereas the lowest uptake (16.9 mg pot^{-1}) was observed in the control. Mean S uptake increased progressively from 20.6 mg pot^{-1} at 0 kg/ha sulphur to 47.3 mg pot^{-1} at 60 kg/ha sulphur, which reflects the improved availability and oxidation of applied sulphur. Soil application of SOB consistently enhanced S uptake over seed treatment and no-SOB treatments. The effects of sulphur, SOB and their interaction on kernel S uptake were significant.

Oil content in groundnut kernels increased steadily with higher sulphur levels and SOB application (Table 3). Across sulphur levels, soil application of SOB recorded the highest oil content, followed by seed treatment, while the lowest values were observed under no-SOB application. Among the sulphur levels, the maximum oil content (51.3 %) was achieved at 60 kg S ha^{-1} with soil application of SOB, whereas the minimum (43.2 %) was recorded in the absolute control. The mean oil content increased from 45.4 % at 0 kg/ha sulphur to 50.4 % at 60 kg S ha^{-1}, which confirms the beneficial role of sulphur in enhancing oil biosynthesis. Results clearly indicated that the main effects of sulphur and SOB, as well as their interaction, were statistically significant. Overall, the combination of higher sulphur levels with SOB application resulted in the greatest improvement in oil content.

Sulphur application in the range of 20–60 kg ha^{-1} has been shown to significantly enhance crop growth, yield, nutrient uptake and overall economic returns in oilseed crops (9). Adequate sulphur availability enhances plant growth by sustaining essential physiological functions and this relationship has been well documented in previous research (10, 11). In addition, oil content is directly influenced by sulphur availability. Earlier research has likewise demonstrated that elemental sulphur, when used as a sulphur source, enhances groundnut yield (12).

Influence of sulphur and SOB on black gram as a residual crop

Blackgram seed yield was significantly influenced by the residual effect of sulphur and SOB application (Table 4). Seed yield increased steadily as the sulphur level increased. The control treatment recorded a lower mean yield of 20.8 g pot^{-1}, while the application of 60 kg S ha^{-1} produced a higher mean yield of 31.3 g pot^{-1} and was on par with S at 40 kg ha $^{-1}$. Among the SOB treatments, soil application consistently yielded higher than seed treatment and no-SOB application. At 60 kg S $^{-1}$, seed yield was 32.3 g $^{-1}$ with soil-applied SOB, compared with 30.4 g pot^{-1} under no-SOB application. Statistical analysis confirmed the significance of sulphur, SOB and their interaction. Overall, the residual sulphur together with SOB application improved the productivity of blackgram. It has been reported that sulphur fertilization enhances the yield of blackgram (13) and this outcome has been confirmed by subsequent studies (14). Similar results have been documented in a mustard–rice cropping sequence, demonstrating that the beneficial effects of sulphur are consistent across diverse cropping systems (15).

Table 3. Effect of sulphur levels on oil content in groundnut CO 7

S levels \ SOB	Oil content (%)			
	S1 No SOB	**S2 Seed treatment with SOB**	**S3 Soil application of SOB**	**Mean**
E1-0	43.2	45.3	47.7	45.4
E2-20	45.6	47.2	48.8	47.2
E3-30	47.1	48.2	49.5	48.3
E4-40	48.5	49.9	50.9	49.8
E5-50	49.0	50.2	51.0	50.1
E6-60	49.2	50.8	51.3	50.4
Mean	47.1	48.6	49.9	
		SEd	**CD (p = 0.05)**	
E		0.26	0.53	
S		0.20	0.41	
ExS		0.43	0.87	

Table 4. Effect of sulphur levels on yield of the residual crop

S levels (Kg S ha^{-1}) / SOB	Blackgram seed yield (g/pot)			
	S1 No SOB	S2 Seed treatment with SOB	S3 Soil application of SOB	Mean
E1-0	19.9	21.0	21.5	20.8
E2-20	23.7	25.7	26.1	25.2
E3-30	25.3	28.0	28.6	27.3
E4-40	27.2	29.9	30.6	29.2
E5-50	29.8	31.4	32.1	31.1
E6-60	30.4	31.3	32.3	31.3
Mean	24.4	26.6	27.1	
		SEd	**CD (p = 0.05)**	
E		1.0	2.2	
S		0.6	1.2	
ExS		1.9	3.9	

Effect of sulphur and SOB application on nutrient uptake in blackgram

Nitrogen and sulphur uptake by blackgram were significantly influenced by sulphur levels and SOB application (Table 5). Nitrogen uptake increased progressively with higher sulphur application, ranging from 573 mg pot^{-1} in the control to 988 mg pot^{-1} at 60 kg S ha^{-1} combined with soil application of SOB. Across the SOB treatments, soil application consistently recorded higher nitrogen uptake than seed treatment and no-SOB application. The mean nitrogen uptake increased from 605 mg pot^{-1} in the control to 954 mg pot^{-1} at 60 kg S ha^{-1} , which indicated a strong residual effect of sulphur and the synergistic role of SOB.

A similar pattern was observed for sulphur uptake, which increased from 31.8 mg pot^{-1} in the control to 93.7 mg pot^{-1} under 60 kg S ha^{-1} with soil-applied SOB. The mean sulphur uptake under soil application of SOB (76.7 mg pot^{-1}) was higher than seed treatment (67.4 mg pot^{-1}) and no-SOB application (56.7 mg pot^{-1}). Statistical analysis confirmed that the main effects of sulphur and SOB, as well as their interaction, were significant for both nitrogen and sulphur uptake. These results highlight that integrated sulphur management with the SOB application effectively enhanced nutrient uptake in blackgram.

The increase in nutrient uptake may be attributed to efficient nutrient utilization through a well-developed root system, which led to higher yields and enhanced nitrogen concentration due to the synergistic effect of sulphur application. A similar result has been reported in earlier studies (16). In plants, sulphur (S) and nitrogen (N) play a central and synergistic role in protein synthesis and the availability of these nutrients is highly interrelated. The sulphur requirement and its metabolism are closely linked to nitrogen nutrition, while the sulphur status of the plant strongly influences nitrogen metabolism. Similar to the connection between carbon and nitrate assimilation, the S and N assimilatory pathways are interconnected, with the availability of one element regulating the other (1). The residual influence of integrated sulphur management led to a gradual increase in nitrogen and sulphur uptake in blackgram, consistent with earlier findings on comparable nutrient uptake interactions (17).

Sulphur availability in post-harvest soil

Post-harvest soil analysis showed that sulphur and SOB treatments had an evident influence on available S (Table 6). The lowest S level of 6.2 ppm occurred in the control plots without sulphur or SOB, whereas the highest value of 8.8 ppm was observed at 60 kg S ha^{-1} with soil-applied SOB. Soil application of SOB resulted in greater S availability than seed treatment or no-SOB application at every sulphur level. The mean available S increased from 6.5 ppm in untreated soil to 8.3 ppm at the highest sulphur dose, which reflected the residual contribution of applied sulphur and the improved mineralization associated with SOB activity. The effects of sulphur, SOB and their interaction were statistically significant and this outcome shows that integrated sulphur management can maintain higher soil S reserves for the succeeding crop.

Effectiveness of elemental sulphur as a sulphur source

Elemental S-based fertilizers are among the most concentrated sources of sulphur. S-bentonite fertilizers are formulated to enhance the efficiency of granular elemental sulphur products by adding about 10 % swelling clay, such as bentonite, by weight. When applied to soil, the bentonite absorbs moisture and causes the fertilizer granules to swell and disintegrate into fine sulphur particles particles that undergo rapid oxidation to form sulphate (SO_4^{2-}). The use of elemental sulphur to lower soil pH and reclaim sodic and

Table 5. Effect of sulphur levels on N and S uptake of blackgram

S levels Kg S ha^{-1} / SOB	Blackgram N uptake (mg/pot)				Blackgram S uptake (mg/pot)			
	S1 No SOB	S2 Seed treatment with SOB	S3 Soil application of SOB	Mean	S1 No SOB	S2 Seed treatment with SOB	S3 Soil application of SOB	Mean
E1 -0	573	611	630	605	31.8	39.9	45.2	39.0
E2-20	692	758	775	742	45.0	56.5	65.3	55.6
E3-30	744	832	855	810	53.1	67.2	77.2	65.8
E4-40	802	891	921	871	62.6	74.8	85.7	74.4
E5 -50	897	955	979	944	71.5	81.6	93.1	82.1
E6-60	918	955	988	954	76	84.5	93.7	84.7
Mean	771	834	858		56.7	67.4	76.7	
		SEd	**CD (p = 0.05)**			**SEd**	**CD (p = 0.05)**	
E		35	72			5.5	11.2	
S		20	42			3.0	6.2	
ExS		52	105			12.3	25.2	

Table 6. Effect of sulphur levels on S availability in post harvest soil

S levels / SOB	Available S (ppm)			
	S1 No SOB	S2 Seed treatment with SOB	S3 Soil application of SOB	Mean
E1-0	6.2	6.5	6.7	6.5
E2-20	6.5	7.1	7.5	7.0
E3-30	7.2	7.5	8.1	7.6
E4-40	7.4	7.6	8.3	7.8
E5-50	7.5	8.0	8.6	8.0
E6-60	7.9	8.3	8.8	8.3
Mean	7.0	7.5	7.9	
		SEd	**CD ($p = 0.05$)**	
E		0.4	0.9	
S		0.2	0.5	
ExS		1.1	2.3	

calcareous soils is well established. Its importance as a fertilizer source has increased in recent years because the availability of other sulphur-based fertilizers has become limited. Elemental sulphur is widely incorporated into high-analysis bulk blend formulations because it provides sulphur in a physical form that readily converts to sulphate (SO_4^{2-})once applied to the soil.

As the oxidation rate of these sulphur sources varies during the first growing season after application, they should be mixed into the soil before planting. Continuous use of elemental sulphur fertilizers gradually increases the population of sulphur-oxidizing microorganisms, resulting in an enhanced rate of sulphate (SO_4^{2-}) formation. This provides an alternative sulphur source for crops and reduces reliance on gypsum.

In calcareous soils, elemental sulphur offers a dual advantage as it functions as a nutrient source and also helps in reducing soil pH. The soil used in the present investigation falls within the range of slight calcareousness, so elemental sulphur serves both as a fertilizer and as an amendment. Furthermore, elemental sulphur is gaining recognition as an effective amendment for soils contaminated with heavy metals. Studies have shown that applying sulphur in elemental form significantly reduces the proportion of acid-extractable Cd and increases the proportion of residual Cd, which in turn lowers diethylenetriaminepentaacetic acid -extractable cadmium (DTPA-Cd) content and effectively limits Cd uptake by plants (18). In Cd-contaminated soils, sulphur serves as an effective soil conditioner, supporting plant growth by decreasing Cd availability.

Role of sulphur-oxidizing bacteria in sulphur management

Microbial oxidation of sulphur is the process by which microorganisms oxidize sulphur to synthesize their structural components. Chemo-lithotrophic microorganisms primarily use the oxidation of inorganic compounds to obtain energy for survival, growth and reproduction. Certain inorganic forms of reduced sulphur, mainly sulfide and elemental sulphur, can be oxidized by chemo-lithotrophic sulphur-oxidizing prokaryotes.

Sulphur-oxidizing bacteria play a significant role in the biogeochemical cycling of sulphur. When elemental sulphur is applied, the presence of SOB becomes essential because plants absorb sulphur only in the sulphate form and this conversion depends on microbial oxidation. The importance of sulphur-oxidizing bacteria in plant sulphur nutrition has been well documented. The use of organic manures, such as vermicompost, further enhances the population of these beneficial microbes (19). In addition, previous studies have highlighted the role of SOB in sulphur oxidation and the resulting improvement in plant productivity (20). In contrast, others have reported their positive influence on sulphate transformation in oilseed crops (21).

Conclusion

Sulphur is essential to primary plant metabolism and plays a key role in antioxidative defence and protection against diverse abiotic stresses. Like other macronutrients, sulphur is indispensable for sustainable soil fertility management, enhancing crop productivity and improving the yield of oilseed and legume crops. Elemental sulphur, when applied to calcareous soil, can help reduce soil pH while simultaneously supplying plant available sulphur, thereby serving as an alternative to gypsum. In the present study, sulphur application of 60 kg ha^{-1}as elemental sulphur along with soil application of SOB at 2 kg/ha significantly influenced pod yield, kernel yield, nutrient uptake and oil content of groundnut in soils of low S status and was on par with S at 40 kg ha^{-1}. Moreover, the residual blackgram crop exhibited increased yield and enhanced sulphur uptake under the same treatment conditions. Post-harvest availability of sulphur has also significantly increased in the sulphur applied treatments. In a cropping sequence, the elemental sulphur applied to the first crop can also meet the sulphur requirement of the succeeding crop, thereby eliminating the need for an additional application. Hence, sulphur application is highly advantageous in groundnut-blackgram cropping sequence and conjoint application of elemental sulphur and SOB demonstrates consistently positive effects.

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Authors' contributions

MRL carried out the pot culture study and drafted the manuscript. RI, GS, RR and KS participated in the design of the study and performed the statistical analysis. SK, TB, VA, JB, MG, VS and GP participated in review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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