ANSF
APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
14(3), 1009 - 1015 (2022)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effect of Bio sulphur granules (BSG) as fertilizer ingredient on different fractions of sulphur in calcareous soil cultivated with blackgram (Var.VBN-8)

G. Akila
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), India
S. Thiyageshwari*
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), India
D. Selvi
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), India
R. Anandham
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), India
M. Djanaguiraman
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641003 (Tamil Nadu), India

*Corresponding author. Email: thiyageshwari@gmail.com

Article Info
https://doi.org/10.31018/jans.v14i3.3702
Received: July 7, 2022
Revised: August 24, 2022
Accepted: August 29, 2022

How to Cite
Akila, G. ***et al.*** (2022). Effect of Bio sulphur granules (BSG) as fertilizer ingredient on different fractions of sulphur in calcareous soil cultivated with blackgram (Var.VBN-8). *Journal of Applied and Natural Science*, 14(3), 1009 - 1015. https://doi.org/10.31018/jans.v14i3.3702

Abstract
The purpose of this study was to examine the various sulphur (S) fractions in experimental pot calcareous soil treated with Bio sulphur granules (BSG) in order to assess the impact of granular sulphur fertilization in S deficient calcareous soil using blackgram (Var. VBN-8) as a test crop.Factorial randomized block design with ten treatments (T_1- Absolute control;T_2-Recommended dose of NPK and S (Control);T_3-Soil test based NPK; T_4-T_3 + S as Elemental Sulphur @ 40 kg S/ha; T_5-T_3 + S as BSGI@ 40 kg S/ha; T_6-T_3 + S as BSGII@ 40 kg S/ha;T_7-T_3 + Vermicompost @ 4 t ha^{-1}; T_8-T_4 + Vermicompost @ 4 t ha^{-1};T_9- T_5 + Vermicompost @ 4 t ha^{-1}; T_{10}- T_6+ Vermicompost @ 4 t ha^{-1}) replicated thrice and 5 pots were maintained for each replication. The results of this study revealed that there was an upward trend in all S fractions in every treatment (T_1 to T_{10}), in the following order: organic > inorganic > water soluble > exchangeable S. The pot that received vermicompost coupled with BSG II (T_{10}) (ES@ 40 kg ha^{-1} and *Methylobacteriumthiocyanatum*VRI7-A4 as S source) was found to have the greatest S-fraction and was higher than other treatments. Therefore, using BSG II in conjunction with vermicompost is necessary to preserve the availability of S nutrients in calcareous soil and increase the solubility of nutrients through S-oxidation.

Keywords: Bio sulphur granules, Black gram, Calcareous soil, Elemental sulphur, Sulphur oxidizing bacteria

INTRODUCTION

Calcareous soils are common in the arid areas of the earth (FAO, 2016), occupying >30% of the earth's surface, and their $CaCO_3$ content varies from just detectable up to 95% (Taalab *et al.,* 2019).In India**,** the estimated area of calcareous soils is 228.8 m ha, covers 69.4% of the country's total geographical area (TGA), and spreads over 38 out of 60 agro-ecological sub-regions (Pal *et al*., 2000). High soil pH and excessive calcium carbonate ($CaCO_3$) content are significant problems in calcareous soils, which reduce nutrient availability to crops, especially phosphorus and micronutrients, and consequently affect the growth and yield of crops (Wright *et al*., 2012). Sensible fertilizer or amendments can improve nutrient balance in calcareous soil, increasing crop yield and fertilizer use efficiency. One method that has been used to boost the availability of pH-sensitive nutrients in calcareous soils is pH modification. The most prevalent and economical acidifying substance is sulphur. Elemental sulphur (ES) application has been recommended to reduce the calcareous soil's pH and consequently increase crop nutrient availability (Amin and Mihoub, 2021). Biochemical oxidation of ES produces sulphuric acid, which decreases the soil pH and solubilizes $CaCO_3$ in alkaline calcareous soil (Besharati, 2017).

Pulses are particularly sensitive to S deficiency, giving low quality and lower yields. S plays a crucial role in chlorophyll and oil synthesis. Plants only uptake sulphur in sulphate form (SO_4^{2-}) and reduce it to form S containing amino acids and other compounds (Dhani, 2020). Most of the S in soil environments (95% of total S) is bound to organic molecules and, therefore, not directly plant available. S oxidizers enhance the rate of natural oxidation of S and speed up the production of SO_4^{2-}, making them available to plants at their critical stages, consequently resulting in increased plant yield (Youssif *et al*., 2015). The most crucial stage of the S cycle, which increases soil fertility, is S oxidation. S oxidation in the soil is significantly influenced by Sulphur Oxidizing Bacteria (SOB). Although the sulphate fraction (inorganic) typically makes up less than 5% of the total S in soil, it is the form of S that is absorbed by plant roots. Sulphate can be found in soil as either a water-soluble or an adsorbed form, depending on the soil's properties like pH and the amount of iron oxide/ hydroxide present. While it is in organic form in the humid zone, sulphate salts of calcium, magnesium, sodium, and potassium predominate in arid soils. However, due to co-precipitation with $CaCO_3$, sulphate may also be present in calcareous soils in the insoluble form (De Bona and Monteiro, 2010).

The soil microbial biomass, which has the highest capacity for both mineralization and subsequent changes in the oxidation state of sulphur, is solely responsible for the transport of sulphur between the inorganic and organic pools. While oxidation-induced acidity helps to solubilize plant nutrients that co-precipitate with $CaCO_3$ and improves alkali soils, it also produces sulphate, which plants can use. (Chapman, 1990). ES and two different SOB (*Pandoraea thiooxydans* ASTB16 and *Methylobacterium thiocyanatum*VRI7-A4) are used to develop bio sulphur granules. Comparatively to seed-applied inoculants, whether in a powder or liquid formulation, granular inoculants applied to soils boosted seed output (Bashan *et al*., 2014). Granular > Powder > Liquid > Uninoculated is the order in which the formulation of inoculants has the most significant impact on nodule number, nitrogen build-up, and N_2 fixation (Clayton *et al.,* 2004). Application of clay-based pellet formulation of *Thiobacillus* to S-deficient soil increased groundnut growth, nodulation, and yield (Anandham *et al.,* 2007). *Coccobacilli* bacteria pellet formulation based on bentonite clay applied to calcareous soil of Kuwait improved plant growth and nutrient concentration of Alfalfa plants(Almatawah, & Al-Surrayai, 2019). There is substantial information available on S nutrition and SOB in relation to blackgram growth (Vidyalakshmi and Sridar, 2007). However, the data related to S nutrition and SOB on S fractions in calcareous soils is insufficient under blackgram cultivation. Accordingly, the present study was formulated with the following objectives (i) To develop bio-sulphur granular inoculants with elemental sulphur and sulphur oxidizing bacteria and (ii) to ascertain the fate of applied BSG on the distribution of various sulphur fractions in calcareous soil used for growing blackgram.

MATERIALS AND METHODS

Growth conditions of SOB strains and development of Bio sulphur granules

Bio sulphur granules were created by combining ES, rice gruel (used as an adhesive), and SOB strains. For mass culture, strains of VRI 7-A4 (*Methylobacterium thiocyanatum*) and ASTB 16 (*Pandoraea thiooxydans*) were routinely cultured in nutrient broth (Himedia, India) for 48 h at 30 °C under shaking conditions (120 rpm). ES was powdered, sieved through a fine mesh sieve (<0.5 mm), and sterilized. For 100 g of powdered elemental S, 250 ml of freshly prepared culture (1×10^8 cells/ml) were mixed and granulated by a pressure-less manual mixing method under aseptic conditions. By mechanical interlocking and cementing, ES powder, bacterial strains, and rice gruel were combined and united into agglomerates (granules). Granulates of varied sizes are produced through manual preparation. Particle sizes ranging from 0 to 2 mm were separated using a 2 mm sieve. As a result, two separate bio-sulphur granules, *P. thiooxydans* ASTB 16 (BSG-I) and *M. thiocyanatum* VRI 7-A4 (BSG-II), were created utilizing two different sulfur-oxidizing bacteria. The efficacy of these two granules was tested in pot experiments.

Particulars of pot experiment

A pot experiment was conducted in the Soil Science and Agricultural Chemistry Department, Tamil Nadu Agricultural University, Coimbatore district, with blackgram variety VBN-8 as a test crop in S deficient calcareous soil to study the sulphur fractions. The soil chosen for the study was sandy clay loam in texture having deficient in S, with alkaline soil reaction (pH-8.3), non-saline (EC-0.03dSm^{-1}), calcareous (free $CaCO_3$-13.5%) and having low available nitrogen (140 kg ha^{-1}) and phosphorus (10.8 kg ha^{-1}), medium in organic carbon content (0.58%) and available potassium (252 kg ha^{-1}).

Ten treatments were replicated thrice in a factorial randomized block design (FRBD) using different treatment combinations mentioned in Table 1.

Nitrogen (N), phosphorous (P), and potassium (K) were applied at rates of 25, 50, and 25 kg ha^{-1}, respectively, and the treatment regimen dictated the use of vermin compost, BSG-I, and II were thoroughly mixed to the soil. The experimental soil texture was sandy clay loam, and the black gram VBN 8 variety's targeted yield (T) was 900 kg/ha. The available NPK (SN, SP & SK) in the soil was minimal, according to the results of the initial soil analysis. Initial soil characteristics of experimental soil are listed below (Table 2). For Soil Test based Crop Response (STCR) treatments, fertilizer doses are determined using an existing Fertilizer Prescription Equation (FPE). The Fertilizer Prescription Equation employed in this investigation was

FN = 10.84T-0.39SN ---------- (1)

FP_2O_5 = 7.23T-1.00SP ---------- (2)

FK_2O = 5.20T-0.04SK ---------- (3)

Entered these available nutrient statuses and targeted yield into the STCR equation, and NPK was applied depending on the requirement. For STCR, NPK +vermicompost @ 4 t ha^{-1} treatments (65% moisture, 1.42, 1.21, 1.13 % N, P, K) were applied in addition to the calculated fertilizer doses from FPE. All fertilizers and sulphur application calculations are based on the weight of the pots (10 kg). The bottom of the pots has drainage holes on the side. Filled the pots with ten seeds at first and trimmed the plants to 5 per pot after two weeks.

Data acquisition

Soil samples were collected treatment-wise from the pot periodically (30^{th}, 45^{th}, and 60^{th}days after sowing (DAS)), processed, and analyzed for different fractions of sulphur by sequential extraction as outlined by (Azmi and Seema, 2018).

Table 1. Treatment details for pot soil experiments

T_1	:	Absolute control (without NPK &S)
T_2	:	NPK+S (Control) (RDF*)
T_3	:	STCR based NPK
T_4	:	T_3 + Sulphur as Elemental Sulphur @ 40 kg S/ha
T_5	:	T_3 + Sulphur as Bio Sulphur Granules I@ 40 kg S/ha
T_6	:	T_3 + Sulphur as Bio Sulphur Granules II@ 40 kg S/ha
T_7	:	T_3 + Vermicompost @ 4 t ha^{-1}
T_8	:	T_4 + Vermicompost @ 4 t ha^{-1}
T_9	:	T_5 + Vermicompost @ 4 t ha^{-1}
T_{10}	:	T_6 + Vermicompost @ 4 t ha^{-1}

RDF*- 25:50:25 kg of N, P_2O_5, K_2O ha^{-1}, and 40 kg S ha^{-1}

Water soluble or plant available sulphur

First, distilled water was used to extract the soil sample at a ratio of 1:10 (w/v). Next, each sample was centrifuged for 10 minutes at 10,000 rpm after 30 minutes of shaking. Pipette out precisely 5 ml of the aliquot into a 25 ml volumetric flask, and then add 10 ml of sodium acetate-acetic acid buffer (pH 4.8), 1 ml of gum acacia solution, and 1 g of $BaCl_2$. With distilled water, the volume was filled and thoroughly shaken. After setting the meter to zero per unit absorbance with the blank, read the absorbance of this solution in a UV-VIS spectrophotometer at 420 nm wavelength. Using the standard curve, the sulphate concentration was estimated (Azmi and Seema, 2018).

Exchangeable or adsorbed sulphur

Following the centrifugation, the samples were extracted with 0.032 M NaH_2PO_4 at the 1:10 (w/v) ratio. After 30 minutes of shaking, each sample was again centrifuged at 10000 rpm for 10 minutes. The extracted sulphur was estimated using turbidity (Azmi and Seema, 2018).

Inorganic sulphur

Inorganic sulphur was extracted by adding 25 ml of 1% HCl solution to the soil residue obtained from the previous extraction, kept for shaking for 10 minutes, and filtered. Finally, the soil was leached with distilled water for free of chloride. After extraction of the S fraction, sulphur was estimated turbidometrically (Azmi and Seema, 2018).

Occluded /precipitated sulphur

The residue from the previous fraction was extracted with 1M HCl. The extraction ratio was 1:20 (w/v) ratio. After 60 minutes of shaking, the samples were centrifuged for 10 minutes at 10000 rpm. The extracted sulphur was estimated using turbidity (Azmi and Seema, 2018).

Total sulphur

The total sulphur content was determined separately by the acid digestion method as per the procedure given by Chesnin and Yien (1951). First, five g of finely ground soil was mixed with 3 ml of 69% Nitric acid and heated in a steam bath. Then, 3 ml of 60% Perchloric acid and 7 ml of Phosphoric acid were added and heated in a sand bath at 190-210ºC until white fumes were visible. Finally, two ml of 37% Hydrochloric acid were added after cooling and heated again until white fumes were visible. The digest was transferred quantitatively, and the volume was adjusted to 100 ml using 1N HCl and analyzed using turbidimetry.

Statistical analysis

The data obtained from the experiment were subjected to statistical analysis using AGRESS software version 7.01. In addition, the treatment means were compared using Tukey's post hoc test at a 0.05 probability level.

RESULTS AND DISCUSSION

This study gives information to assess the impact of developed BSG on various sulphur fractions in calcareous soil. According to the analysis of variance, applied sulphur enters into various forms, mainly organic and inorganic, water-soluble, and sulphate. All applied S significantly (P=.05) enhanced all fractions of S in calcareous soil on 30, 45th, and 60DAS, thus the maximum was recorded in inoculation of *M. thiocyanatum*VRI7-A4as BSG II @ 40 kg S ha^{-1}+ vermicompost (4t ha^{-1}) applied treatment (T_{10}) in a pot experiment.

Water soluble sulphur fraction (WS-S)

Water soluble fraction was recorded on the pot experiment's 30th, 45th, and 60th day. This fraction exerts a significant interaction on S treatment and increases with the advancement of blackgram growth. The highest mean value of water soluble-S was recorded in T_{10} (from 16.92 to 18.03 mg kg^{-1}), and this was on par with T_6 having a mean value of (15.89 to 17.9 mg kg^{-1}) and the lowest mean value (from to 6.23 to 4.73 mg kg^{-1}) was observed in control (Fig.1). This Mean value of WS-S accounted for around 4.9 to 14.6 % of total S which is shown in Table 3. Over time, the WS-S content in T_{10} was enhanced by BSG II (40 kg S ha^{-1}) as S source and vermicompost(4t ha^{-1}) and remained higher than in other treatments. This may result from SOB-assisted ES oxidation and the release of S from organic sources. The poor outcomes with ES application could be because S oxidation and subsequent mineralization by the sulphur oxidizing bacteria take time. Similar results were also reported by (Karimizarchi*et al*., 2014), who discussed that concerning ES, S immobilization is the primary process at the beginning of S-oxidation. This is followed by S mineralization and the production of sulphates as inorganic soluble sulphur forms (Thirunavukarasu and Subramanian, 2015).

Exchangeable-S/ Adsorbed sulphur fraction (EX-S)

The plant available S fractions in the soil include the water soluble-S, adsorbed sulphur, and inorganic sulphur of the total sulphur. Among the available sulphur fractions, the trend observed was inorganic S > water-soluble sulphur> exchangeable sulphur. It was also noted that there was a significant (P=.05) increase in the EX-S fraction. This Mean value of EX-S accounts for around 4.62 to 9.63 % of total sulphur, as shown in Table 3. These observations follow the findings of earlier researchers (Clarson and Ramaswami, 1992), who reported an increasing trend observed in available S with increasing levels of applied sulphur and crop growth. Among the treatments ($T_1$to T_{10}), application of ES @ 40 kg S ha^{-1} + *M. thiocyanatum*VRI7-A4 (T_6) registered the highest soil EX-S fractions significantly till 45 DAS, and later it was on par with T_{10} @ 40 kg S ha^{-1} +*M.thiocyanatum*VRI7-A4 + Vermicompost (4t ha^{-1}) till 60 DAS (Fig.2). Decrease in the available S (WS-S+EX-S) content initially was due to higher removal of native S by the crop as the biomass production was reported to be higher initially (Islam *et al.,* 2012). Later gradual build-up was due to lower biomass production and, in turn, lower uptake of native S compared to the rate of S mineralization from the soil (Lavanya *et al.,* 2019).

Table 2. Physicochemical properties of initial soil-pot experiment

1. Physical properties	**Eastern block, TNAU, Coimbatore**
Bulk density (Mg m^3)	1.24
Partical density (Mg m^3)	1.66
Porosity (%)	6.57
2. Mechanical analysis	
Coarse sand (%)	29.9
Fine sand (%)	28.7
Silt (%)	15.4
Clay (%)	25.4
Textural class	Sandy clay loam
3. Electrochemical properties	
pH	8.3
EC(dS m^{-1})	0.03
CEC (c mol (p+) kg^{-1})	34.7
4. Chemical properties	
Organic carbon (%)	0.58
Available Nitrogen(kg ha^{-1})	140
Available Phosphorus (kg ha^{-1})	10.8
Available Potassium (kg ha^{-1})	252
Available Sulphur (mg kg^{-1})	2.5
DTPA extractable Fe (mg kg^{-1})	5.10
DTPA extractable Zn (mg kg^{-1})	1.61
DTPA extractable Mn (mg kg^{-1})	0.99
DTPA extractable Cu (mg kg^{-1})	0.12
Free $CaCO_3$ (%)	13.5

Inorganic S fraction

The Inorganic S content in soil was significantly (P=.05) influenced by S nutrition (Fig. 3). ES with *M.thiocyanatum* VRI7-A4 (T_6) increased the inorganic S. Among the treatments (T_6 toT_{10}), the highest inorganic-S content of 21.25 mg kg^{-1} was recorded in the soil applied with 40 kg ha^{-1} of ES with SOB (T_6), which is on

Table 3. Percentage of various sulphur fractions concerning total sulphur in post-harvest surface soil (on 60th day) of Blackgram (Var-VBN 8)

Fractions / Treatments	Water soluble S (%)	Exchangeable S (%)	Inorganic S (%)	Organic S (%)
T_1- Absolute control	5.40	4.82	9.39	38.55
T_2- NPK+S (Control)	4.92	4.62	8.28	34.24
T_3 - Soil test-based NPK	9.59	6.89	13.73	38.47
T_4 - T_3+ES	10.21	7.03	14.07	40.12
T_5 - T_3+BSG I	14.17	9.08	16.16	49.15
T_6 - T_3+BSG II	14.64	9.63	17.30	52.08
T_7 - T_3+Vermicompost	11.22	7.98	14.49	40.48
T_8 - T_4+Vermicompost	11.75	8.07	14.43	42.87
T_9 - T_5+Vermicompost	13.95	9.39	16.01	50.38
T_{10} - T_6+Vermicompost	14.45	9.44	16.79	53.74
Mean	11.03	7.69	14.06	44.01

par with 40 kg S ha^{-1} in the form of ES with *M.thiocyanatum* VRI7-A4+ vermicompost (T_{10}) and the lowest content of 8.22 mg kg^{-1} was recorded in absolute control (T_1) on 60th days after sowing. Among different fractions, the inorganic S fraction registered about 8.28 to 17.3% (Table 3). The addition of S fertilization with oxidizing micro organism to calcareous soil favoured solubilization of the sulphate that was co-precipitated with $CaCO_3$, and the solubilized sulphate was partly transformed into soluble inorganic form (Hu *et al*., 2005). The results conformed with the observations recorded by Sharma *et al*. (2014); they showed that zero fertilization led to a decline in the levels of all S forms, while application of S-containing fertilizer and organics increased it over control. This might be due to the release of S from inorganic and organic S sources applied to different treatments, and the treatments which recorded lower inorganic S were due to continuous crop removal without the addition of any S source and conversion of the inorganic form of S to SO_4^{2-}-S (Roshini*et al*., 2021).

Organic sulphur fraction

The organic-S fraction is the slowly available S fraction of the total sulphur. Over time, this fraction slowly releases SO_4^{2-} and serves as a soil available S pool. The extent of increase over the days was found maximum in T_{10} (from 65.98 to 67.06 mg kg^{-1}), which received both vermicompost and BSG II, followed by T_6 (from 61.54 to 63.98 mg kg^{-1}) (Fig.4). This Mean value of organic-S fraction accounts for around 34.24 to 53.74 % of total sulphur which is shown in Table 3. It was also found that; the soil organic-S fraction was higher than all available sulphur fractions. This might be due to the soil's precipitation of sulphates with calcium and other elements. These results are in close agreement with Balanagoudar and Satyanarayana (1990), who had reported that occluded sulphur or non-sulphate sulphur is mainly made up of sulphate occluded in and adsorbed on carbonates or insoluble sulphur compounds of iron and aluminium in soil which remains unextractable after removal of organic carbon and SO_4^{2-} sulphur (Jat and Yadav 2006; Lavanya *et al.,* 2019).

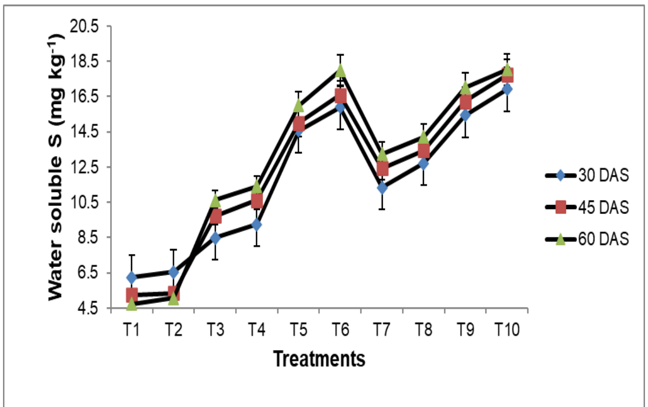

Fig. 1. *Effect of BSG on water soluble sulphur fractions in calcareous soil. (Mean ± SEM = Mean values ± Standard error of means of WS-S)*

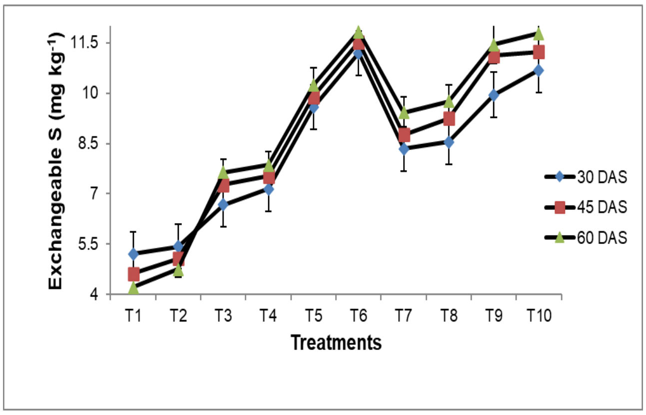

Fig. 2. *Effect of BSG on exchangeable sulphur fractions in calcareous soil. (Mean ± SEM = Mean values ± Standard error of means of Ex-S)*

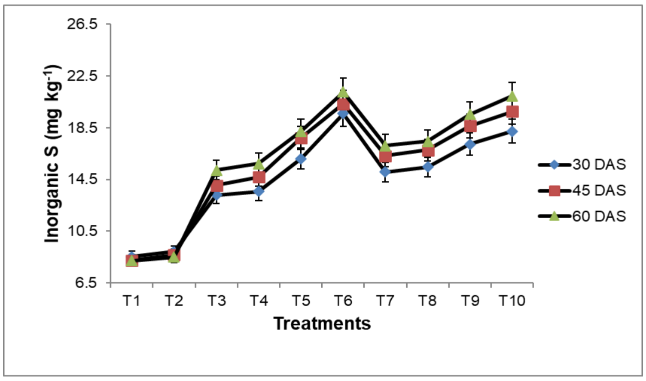

Fig. 3. *Effect of BSG on inorganic sulphur fractions in calcareous soil. (Mean ± SEM = Mean values ± Standard*

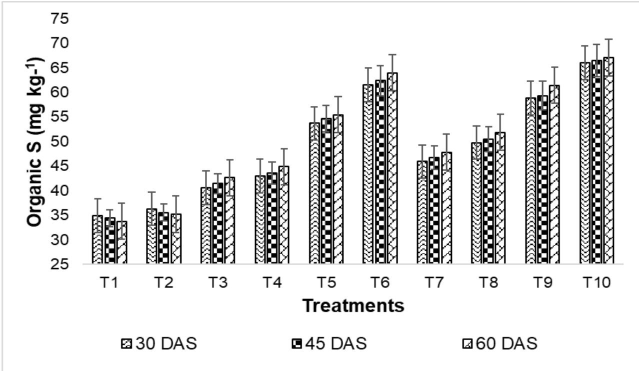

Fig. 4. *Effect of BSG on organic sulphur fractions in calcareous soil. (Mean ± SEM = Mean values ± Standard error of means of organic S)*

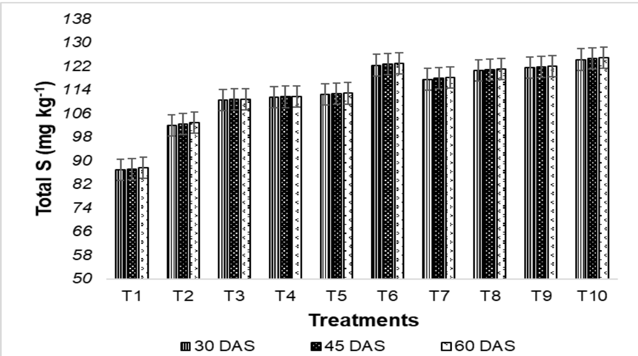

Fig. 5. *Effect of BSG on total sulphur fractions in calcareous soil. (Mean ± SEM = Mean values ± Standard error of means of total S)*

Total sulphur (T-S)

Regarding total sulphur, the effects of sulphur application did not alter the T-S status. The maximum T-S was found in T_{10} (from 124.23 to 124.78 mg kg^{-1}), followed by T_6 (from 122.34 to 122.86 mg kg^{-1}) on 60DAS. The use of organic manure (Vermicompost) and sulphur through S oxidation helped in the build-up of Total S in these treatments (Fig. 5). Das *et al*. (2012) discovered that there was no increase in total S content in manure fertilized treatment. They found the increase to be dependent on the dose of this fertilizer. The results conformed with the findings of Mazur and Mazur (2015) and Gourav*et al*. (2018). Sulphur through SSP helped build Total S in T_2 and T_3 (without S) treatments. An increase in total sulphur content was found to be minimum in treatment which received only control (86.84 to 87.57 mg kg^{-1}) (Fig.5). This might be due to cropping without S in calcareous soil results in the release of S from other S fractions to the available pool for crop uptake as there is equilibrium exists between different fractions of sulphur in soil (Nguyen and Goh, 1990; Roshini *et al*., 2021).

Conclusion

The present study concluded that BSG granules substantially influenced distinct soil fractions in calcareous soil. The different fractions of sulphur were present in the order of organic > inorganic > water soluble> exchangeable sulphur, and the primary form was organic. However, as there is equilibrium between the various fractions of S in the calcareous soil, treatments that involved cropping without sulphur in the soil caused S to be released from other pool fractions available for crop uptake. It was discussed how the availability of different forms of sulphur in calcareous soils was influenced by S oxidation with the help of sulphur oxidizing bacteria under blackgram (var. VBN 8) cultivation. Therefore, the integration of elemental sulphur with sulphur oxidizing bacteria and organic manures is essential for maintaining and sustaining soil fertility in terms of sulphur status for improved blackgram growth in calcareous soil.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Almatawah, Q. A. & Al-Surrayai, T. I. (2019. Development of Biological Sulfur Fertilizer for the Improvement of Desert Soil Fertility in Kuwait.*Research & Reviews: Journal of Ecology and Environmental Sciences,* 7(2), 22-30
2. Anandham, R. Sridar, R. Nalayini, P. Poonguzhali, S. & Madhaiyan, M. (2007). Potential for plant growth promotion in groundnut (Arachis hypogaea L.) cv. ALR-2 by co-inoculation of sulfur-oxidizing bacteria and Rhizobium. *Microbiological Research*, *162*(2), 139-153.Doi:https://doi.org/10.1016/j.micres.2006.02.005
3. Amin, A. E. E. A. Z. & Mihoub, A. (2021). Effect of sulfur-enriched biochar in combination with a sulphur-oxidizing bacterium (Thiobacillus Spp.) on release and distribution of phosphorus in high calcareous p-fixing soils. *Journal of Soil Science and Plant Nutrition*, *21*(3), 2041-2047.Doi:https://doi.org/10.1007/s42729-021-00500-5
4. Azmi, N. Y. & Seema, M. K. (2018). New technique for sequential fractionation of soil sulphur. *International Jour-*

nal of Current Microbiology and Applied Sciences, 3397-3405.

5. Balanagoudar, S. R.& Satyanarayana, T. (1990). Correlations of different forms of sulphur with soil properties and with organic carbon and nitrogen in some Vertisols and Alfisols. *Journal of the Indian Society of Soil Science*, *38* (4), 641-645.
6. Bashan, Y. de-Bashan, L. E. Prabhu, S. R. & Hernandez, J. P. (2014). Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant and Soil*, *378*(1), 1-33. Doi: https://doi.org/10.1007/s11104-013-1956-x
7. Besharati, H. (2017). Effects of sulfur application and Thiobacillus inoculation on soil nutrient availability, wheat yield and plant nutrient concentration in calcareous soils with different calcium carbonate content. *Journal of Plant Nutrition*, *40*(3), 447-456. Doi:https://doi.org/10.1080/01904167.2016.1245326
8. Chapman, S. J. (1990). Thiobacillus populations in some agricultural soils. *Soil Biology and Biochemistry*, *22*(4), 479-482. Doi: http://doi.org/10.1016/0038-0717 (90)90181-X
9. Chesnin, L. & Yien, C. H. (1951). Turbidimetric determination of available sulfates. *Soil Science Society of America Journal*, *15*(C), 149-151.
10. Clarson, D. & Ramaswami, P. P. (1992). Sulphur balance in soils as influenced by sulphur carriers and crops. *Journal of the Indian Society of Soil Science*, *40*(2), 313-316.
11. Clayton, G. W., Rice, W. A., Lupwayi, N. Z., Johnston, A. M., Lafond, G. P., Grant, C. A., & Walley, F. (2004). Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N2 fixation, and nitrogen partitioning. *Canadian Journal of Plant Science*, *84*(1), 79-88. Doi:https://doi.org/10.4141/P02-089
12. De Bona, F. D. & Monteiro, F. A. (2010). Nitrogen and sulfur fertilization and dynamics in a Brazilian Entisol under pasture. *Soil Science Society of America Journal*, *74* (4), 1248-1258. Doi: http://doi.org/10.2136/sssaj2009.0228
13. Das, K. N. Basumatari, A. and Borkotoki, B.(2012). Forms of sulphur in some rapeseed growing soils of Assam. J. Indian Soc. Soil Sci.,60(1): 13-19.
14. FAO. (2016). FAO Soils Portal: Management of Calcareous Soils. *(accessed 01.04. 16)*.
15. Förster, S. Welp, G. & Scherer, H. W. (2012). Sulfur specification in bulk soil as influenced by long-term application of mineral and organic fertilizers. *Plant, Soil and Environment*, *58*(7), 316-321. Doi: https://doi.org/10.17221/32/2012-PSE
16. Gourav, Sankhyan, N. K.Sharma, R. P. and Sharma, G. D.(2018). Vertical distribution of sulfur fractions in a continuously fertilized acid Alfisol under Maize–Wheat Cropping System. Comm. soil sci. plant analysis., 49(8): 923-933. Doi: http:// doi.org/10.1080/00103624.20 18.1448409
17. Hu, Z. Y. Zhao, F. J.& McGrath, S. P. (2005). Sulphur fractionation in calcareous soils and bioavailability to plants. *Plant and Soil*, *268*(1), 103-109. Doi: http://doi.org/10.1007/s11104-004-0229-0
18. Islam, Muhammad, Saleem Mohsan, Safdar Ali, Rizwan Khalid, and Sher Afzal. (2012). "Response of chickpea to various levels of phosphorus and sulphur under rainfed conditions in Pakistan." *Romanian Agricultural Research* no. 29:175-183.
19. Jat, J. R. and Yadav, B. L. (2006). Different forms of sulphur and their relationship with properties of Entisols of Jaipur district (Rajasthan) under mustard cultivation. J. Indian Soc. Soil Sci., 54(2): 208-212.
20. Karimizarchi, M. Aminuddin, H. Khanif, M. Y. &Radziah, O. (2014). Elemental sulphur application effects on nutrient availability and sweet maize (Zea mays L.) response in a high pH soil of Malaysia. *Malaysian Journal of Soil Science*, *18*(1), 75-86.
21. Lavanya, K. R. Kadalli, G. G. Patil, S. Jayanthi, T. Naveen, D. V.,& Channabasavegowda, R. (2019). Sulphur Fractionation Studies in Soils of Long-Term Fertilizer Experiment under Finger Millet–Maize Cropping Sequence. *International Journal of Current Microbiology and Applied Sciences*, *8*(9), 1334-1345. Doi: http://doi.org/10.20546/ijcmas.2019.809.153
22. Mazur, Z. and Mazur, T. (2015). Influence of long-term fertilization on phosphorus, potassium, magnesium, and sulfur content in soil. Polish J. Environ. Studies, 24(1). Doi: http://doi.org/10.15244/pjoes/29203
23. Nguyen, M. L. and Goh, K. M.(1990). Accumulation of soil sulphur fractions in grazed pastures receiving long-term superphosphate applications. New Zealand Agri. Res., 33 (1): 111-128. Doi: http://doi.org/10.1080/00288233.1990.10430668
24. Pal, D. K. Bhattacharyya, T. & Velayutham, M. (2000). Genesis and Classification of Calcareous Soils of India. In *Proceedings of national symposium September* (pp. 19-22).
25. Roshini, S. Jegadeeswari, D.Chitdeshwari, T. & Sankari, A. (2021). Effect of Different Sulphur Sources on Sulphur Fractionation in a Red Sandy Loam Soil.*International Journal of Plant & Soil Science,* 33(22): 87-95
26. Sharma, U. Subehia, S. K. Rana, S. S. Sharma, S. K. and Negi, S. C. (2014). Soil sulphur fractions and their relationship with soil properties and rice (Oryza sativa L.) yield under long-term integrated nutrient management in an acid Alfisol. Research on Crops, 15(4). Doi: http://doi.org/10.5958/2348-7542.2014.01406.5
27. Thirunavukkarasu, M.& Subramanian, K. S. (2015). 3. Nano-sulphur on biomass, yield attributes, soil microbes and physiological parameters of groundnut. *life sciences leaflets*, *63*, 13-to
28. Taalab, A. S. Ageeb, G. W. Siam, H. S. & Mahmoud, S. A. (2019). Some Characteristics of Calcareous soils. A review AS Taalab1, GW Ageeb2, Hanan S. Siam1 and Safaa A. Mahmoud1. *Middle East J*, *8*(1), 96-105.
29. Vidyalakshmi, R. & Sridar, R. (2007). Isolation and characterization of sulphur oxidizing bacteria. *Journal of culture collections*, *5*(1), 73-77
30. Wright, A. L.Hanlon, E. A. & Rice, R. (2012). Managing pH in the Everglades agricultural soils. Florida Cooperative Extension Service Fact Sheet SL-287. UF/IFAS Electronic Data Information Source (EDIS) Database.
31. Youssif, B. D., Hosna, M. A. F., & Mervat, A. A. T. (2015). Effect of Sulphur and Sulphur oxidizing bacteria on growth and production of garlic (Allium sativum, L.) under saline conditions. *Middle East J. Agric. Res*, *4*(3), 446-459.