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M Thirunavukkarasu
Krishi Vigyan Kendra, Gandhigram Rural Institute-DU, Dindigul, Tamil Nadu, India

KS Subramanian
Department of Nano Science and Technology, TNAU, Coimbatore, Tamil Nadu, India

P Kannan
Dryland Agricultural Research Station, TNAU, Chettinad, Tamil Nadu, India

T Balaji
Agricultural Research Station, TNAU, Virinjipuram, Tamil Nadu, India

Correspondence
M Thirunavukkarasu
Krishi Vigyan Kendra, Gandhigram Rural Institute-DU, Dindigul, Tamil Nadu, India

Response of Nano-Sulphur to the groundnut

M Thirunavukkarasu, KS Subramanian, P Kannan and T Balaji

Abstract
An attempt was made to study the response of nano-sulphur and conventional sulphur in groundnut in completely randomized block design and replicated thrice during 2013-14. The results indicated that nano-sulphur @ 30 kgha^{-1}recorded of 0.76 mg,40.5 mg, 14.9 mg, 3.09 mg root, shoot, kernel and shell sulphur uptake plant^{-1}respectively, whereas conventional sulphur @ 40 kg ha^{-1} registered root, shoot, kernel and shell sulphur uptake of 0.53, 35.8, 11.4 and 2.46 mg plant^{-1}, respectively. The highest pod yield was recorded of 12.4 g plant^{-1}with nano-sulphur application @ 30 kg ha^{-1}when comparison to conventional sulphur @ 40 kg ha^{-1}registered of 10.7 g plant^{-1}. The higher oil, crude protein, methionine, cysteine and total free amino acid content of 48.3%, 27.2%, 3.44 mg 100g protein^{-1}, 1.89 mg 100g protein^{-1} and 46.3 mg plant^{-1} were recorded under nano-sulphur application respectively than rest of the sulphur sources. Finally, study concluded that nano-S @ 30 kg ha^{-1} is sufficient to attain higher sulphur use efficiency with reduction of sulphur fertilizer to the tune of 25% besides augment the soil sulphur reserve without harming the environment.

Keywords: Nano Sulphur, groundnut, amino acids, enzyme, SUE

Introduction
The deficiency of available status of Sulphur in India is about 40-45% of the 1, 35,000 soils analyzed across the country (Tandon, 2011) [36]. Soil registering available S below the critical level (10 mg kg^{-1}) is likely to cause yield and quality of agricultural commodities particularly oil seed crops (Tandon and Messick, 2002) [37]. Deficiency of sulphur is becoming widespread due to continuous use of sulphur free fertilizers, high yielding crop varieties, sulphur oxidation, reduced industrial emissions of sulphur dioxide, soils under constant leaching, surface erosion of the high rainfall tropical areas, fixation, intensive multiple cropping system and high sulphur requiring crops along with the restricted or no use of organic manures have accrued in depletion of the soil sulphur reserve (Meena *et al*., 2013) [25]. The basic idea of the experiment is to enhance the sulphur use efficiency and overcome the sulphur deficiency through nano-sulphur formulation application which can plays important role in reduce the nutrient losses, steady and slow nutrient release in rhizosphere target and increase the sulphur use efficiency of the crops. Groundnut accounts 40 per cent of the area (8 million ha) and 30 per cent of the production (6.25 million tonnes) of the total oil seed grown in India (Agriculture Statistics at A Glance, 2009-10) [1]. Kamdi *et al.* (2014) [17] reported that sulphur is required for synthesis of sulphur containing amino acids such as methionine, cystine and cysteine, proteins and increased oil content in groundnut. Nano-fertilizer may be defined as the nano-particles, which can be directly supply of essential nutrients for plant growth, have higher nutrient use efficiency and can be delivered in a timely manner to a rhizosphere target. Nano-particles can absorb on to the clay lattice thereby preventing fixation while releasing in to the soil solution than can be utilized by plants. The process improves soil health and nutrient use efficiency by crops. Fertilizer particles can be coated with nano-membranes that facilitate in slow and steady release of nutrients thereby reducing loss of nutrients and enhancing its use efficiency of crops (Subramanian and Tarafdar, 2011) [34]. A patented nano-composite consists of N, P, K, micronutrients, mannose and amino acids that increase the uptake and utilization of nutrients by grain crops has been reported (Jinghua, 2004) [15].

Huang and Petrovic (1994) reported that the use of slow release fertilizer increased the yield of golf greens biomass due to the slow release of NH_4 from slow release fertilizer. Liu *et al.* (2006) [23] stated that nano-composites containing organic polymer intercalated in the layers of kaolinite clays can be used as a cementing materials to regulate the release of nutrients from conventional fertilizer. This process increases the nutrient use efficiencies besides preventing environmental hazards.

Malhi *et al*. (2002) [24] revealed that polymer coating of mono ammonium phosphate (MAP) improved plant recovery of fertilizers phosphorus and provided a modest barley grain yield advantage to uncoated MAP. In maize, relatively higher yield response to nano-rock phosphate was obtained as compared to micro-sized rock phosphate (Das, 2011) [6]. Lee *et al.* (2008) [21] indicated that significant uptake of nano-sized copper by mung bean and wheat was observed. Significant uptake, translocation and accumulation of Fe_2O_3nano-particle in the roots and leaves of pumpkin has been reported without any effect on the growth and development of the test species (Zhu *et al.*, 2008) [39].

The hypothesis of the study is revealed that sulphur fertilization is very restricted to crops and its use efficiency hardly exceeds 20% with conventional sulphur fertilizers. This necessitates using slow release nano-fertilizer in crop production system, so that use efficiency can be improved. Considering the importance of S in oilseed crops, the present paper deals with effect of nano-sulphur and conventional sulphur fertilization on Sulphur fractionation in soil, sulphatase activity, sulphur uptake, pod and haulm yield and quality attributes of groundnut.

Materials and Methods

A pot culture experiment was carried out with one control treatment (T_1) and each four levels of conventional sulphur (CS) fertilizer (T_2-10, T_3- 20, T_4-30 and T_5-40 kg S ha^{-1}) and sulphate loaded surface modified nano-zeolite or nano-S fertilizer (T_6 - 10, T_7 - 20, T_8 - 30 and T_9 - 40 kg S ha^{-1})with recommended dose of fertilizer @ 25:50:75 kg NPK ha^{-1}were applied to all treatments, Glass House, Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, during 2013. The experiment was conducted in factorial completely randomized block design with three replications. The initial soil had available sulphur of 9.3 ppm (0.15% $CaCl_2$ extractable sulphur method of Chesnin and Yien, 1950) [5], total sulphur of 246 ppm (HCL extractable method of Hesse, 1971) [10], organic sulphur of 174.6 ppm (Morche, 2008) [26], water soluble sulphur of 5.9 ppm (1:10 (w/v) ratio method of Morche, 2008) [26], exchangeable sulphur of 2.5 ppm (0.032MNaH_2PO_4 extractable method of Morche, 2008) [26], occluded sulphur of 63.1 ppm (1M HCL extractable method of Morche, 2008) [26], soil pH of 7.74 (Potentiometry method of Jackson, 1973), EC of 0.33 dSm^{-1} (Conductimetry method of Jackson, 1973), organic carbon of 0.54 % (Wet chromic acid digestion method of Walkley and Black, 1934) and CEC of 21.5 Cmol $p^{(+)}$ kg^{-1} (Neutral Normal Ammonium Acetate method of Schollenberger and Dreibelbis, 1930). Sulphur uptake was calculated from multiplication of nutrient content and dry matter production.

Total sulphur content of plant was extracted using diacid extract (2:1 Nitric and Perchloric acid) and concentration of sulphur was measured at 420 nm in UV-VIS spectrophotometer as per method suggested by Palaskar *et al*. (1981) [28]. Total free amino acid content of the groundnut kernel was extracted using 80% ethanol and ninhydrin solution added and measured in UV- VIS spectrophotometer at 570 nm (Spies, 1955).Methionine content of the groundnut kernel was determined by hydrolysate with addition of 2N hydrochloric acid, sodium nitroprusside and glycine and read the intensity of red colour using DL-methionine as standard at 520 nm in UV- VIS spectrophotometer (Sadasivam and Manickam, 2008) [30]. Cysteine contents of kernel was determined UV-VIS. spectrophotometrically at 412 nm using Ellman's reagents (DTNB, 5, 5'-dithio-bis (2-nitrobenzoic acid) for blue colour development (Nakamura and Binkley, 1948) [27].

Oil content of groundnut kernel was extracted using petroleum ether in soxhlet extractor and expressed as percentage (Sadasivam and Manickam, 2008) [30]. Total nitrogen content of kernel was estimated by micro-kjeldahl's method in the defatted material as followed by Humphries (1956) [12]. Total nitrogen content was multiplied by the factor 6.25 (Dubtez and Wells, 1968) [8] to get crude protein content. Arylsulphatase activity of soil was analyzed using 0.05 M *p*-nitrophenylsulphate solution. The intensity of the yellow colour was measured in an UV-VIS spectrophotometer at 400 nm. The enzyme activity was expressed in µg *p*-nitrophenol per g of soil per h on dry weight basis at pH 5.8 (Tabatabai and Bremner, 1970) [35].

Fractionations of Sulphur

Sulfur is distributed in soil in five forms such as water soluble, exchangeable, occluded, organic-S and total S and these fractions were estimated at the harvest using standard protocol **(**Morche, 2008) [26]. The soil sample was first extracted with demineralized water at 1:10 (w/v) ratio for water soluble fraction, extracted with 0.032M NaH_2PO_4 at the 1:10 (w/v) ratio for exchangeable fraction, extracted with 1M HCl for occluded fraction and total sulphur of soil was analyzed by barium sulphate precipitation method (Hesse,1971) [10]. A sum total of the water soluble, exchangeable and occluded sulphur in the soil was referred as the inorganic S. The total sulphur was subtracted from inorganic sulphur to get organic sulphur.

Results and Discussion

Sulphur Uptake

Sulfur fertilization through NS (Sulphate loaded surface modified nano-zeolite) and CS (conventional sulphur) increased the uptake of sulphur by root, shoot, kernel and shell at harvest stage of the groundnut. The highest root, shoot, kernel and shell S uptake of 0.76, 40.5, 14.9 and 3.09 mg $plant^{-1}$ were recorded by the application of NS fertilizer @ 30 kg S ha^{-1}, respectively. Same set of measurements in CS fertilization registered S uptake of 0.43, 30.6, 8.37 and 2.15 mg $plant^{-1}$ by the root, shoot, kernel and shell, respectively (Table 1). This might be due to highest availability of sulphur in the soil resulted from applied SMNZ based sulphur fertilizer there by increased uptake of the crop. The increased sulphur uptake by the crop due to slow and steady release of sulphur was occurred even after 816 hours whereas conventional sulphur was exhausted within 384 hours. This suggests that surface modified nano-zeolite based sulphur fertilizer may be used as strategy to regulate the smart release of nutrients that commensurate with crop requirement.This is in confirmation with the views of Kundu *et al.* (2010) [20] who have revealed that in sandy soils of Jodhpur at lower concentration of phosphorus in the form of nano rock phosphate, P recovery was higher as compared to that from KH_2PO_4 whereas the reverse occurred with higher concentration of P of nano particle rock phosphate. Significant uptake of nano-sized copper by the mungbean was observed (Lee *et al*., 2008) [21].

Table 1: Effect nano- sulphur on root, shoot, kernel and shell sulphur uptake of groundnut

S Levels (Kg S ha^{-1})	sulphur uptake (mg plant^{-1})							
	root		shoot		kernel		shell	
	CS	NS	CS	NS	CS	NS	CS	NS
L_{10}	0.32	0.35	22.2	24.4	4.74	5.74	1.49	1.76
L_{20}	0.35	0.40	26.8	32.5	6.38	8.99	1.69	2.20
L_{30}	0.43	0.76	30.6	40.5	8.37	14.9	2.15	3.09
L_{40}	0.53	0.66	35.8	38.1	11.4	13.0	2.46	2.62
Control	0.29		15.3		2.74		0.99	
	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
L	0.009	0.019	0.58	1.22	0.17	0.37	0.04	0.09
F	0.006	0.013	0.41	0.86	0.12	0.26	0.03	0.06
LxF	0.013	0.027	0.81	1.73	0.24	0.52	0.06	0.12

Pod and haulm yield

Among the various treatments, the highest pod yield of 12.4 g plant^{-1}and haulm yield of 26.8 g plant^{-1}were recorded in Nano-S @ 30 kg ha^{-1}fertilized plants. The CS applied @ 30 kg S ha^{-1} registered lower pod and haulm yields of 9.41 and 20.3 g plant^{-1}, respectively, but the yields are significantly higher than control. Pod yields in NS fertilized treatments were higher by 7, 17.3, 31.7 and 6.4% under incremental levels of S fertilization (10, 20, 30 and 40 kg S ha^{-1}) in comparison to corresponding CS fertilization (Fig. 1).The increased pod yield is due to increased sulphur uptake as the result of enhanced availability of essential nutrients in soil due to the application of SMNZ based sulphur fertilizer. This might be due to more accumulation of amino acids and amide substances and their translocation to reproductive organs which influenced growth and yield due to application of sulphur (Dongarkar *et al.*, 2005) [7].

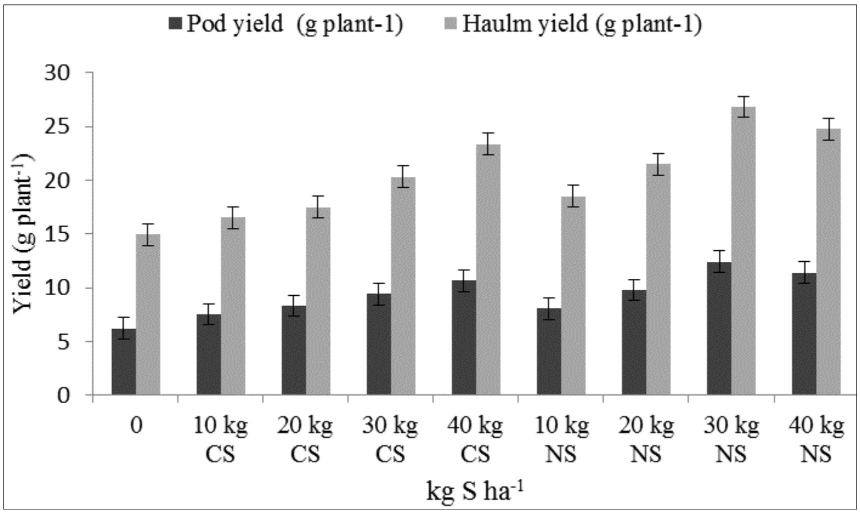

Fig 1: Effect of nano-sulphur on pod and haulm yield of groundnut

Amino Acids

The experimental results showed that application of different levels of nano-sulphur fertilizer significantly influenced the amino acid content of groundnut kernel. Overall, the NS fertilization @ 30 kg S ha^{-1} registered the highest total free amino acid (46.3 mg g^{-1}), methionine (3.44 mg 100 g protein^{-1}) and cysteine content (1.89 mg 100 g protein^{-1}) of groundnut kernel (Table 2). The CS fertilization @ 30 kg S ha^{-1} recorded lower total free amino acid (39.6 mg g^{-1}), methionine (3.16 mg 100 g protein^{-1}) and cysteine content (1.75mg 100 g protein^{-1}). The increased amino acid content in kernel may be due to higher sulphur availability, Owing to its influence on amino acid synthesis in oil seed crops because sulphur is take part in structure of amino acids formation which might be due to sulphur applied as SMNZ, from which sulphur plays important role in chlorophyll formation, increase oil and protein contents, cysteine and methionine contents of groundnut kernel (Kalaiyarasan *et al.*, 2007) [16].

Table 2: Effect nano- sulphur on amino acid content of groundnut

S Levels (Kg S ha^{-1})	Total free amino acids (mg g^{-1})		Methionine (mg 100g protein^{-1})		Cysteine (mg 100g protein^{-1})	
	CF	NF	CF	NF	CF	NF
L_{10}	34.2	36.7	2.84	2.87	1.48	1.52
L_{20}	35.1	38.7	3.08	3.22	1.69	1.74
L_{30}	39.6	46.3	3.16	3.44	1.75	1.89
L_{40}	42.6	44.5	3.29	3.31	1.82	1.83
Control	29.3		2.46		1.25	
	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
L	0.74	1.56	0.06	0.12	0.03	0.07
F	0.52	1.10	0.04	0.09	0.02	0.05
LxF	1.04	2.21	0.08	NS	0.05	NS

Crude protein and oil content

The application of NS fertilization @ 30 kg S ha^{-1} registered higher oil (48.3%) and crude protein (27.2%) contents of groundnut kernels. The CS and NS @ 30 kg S ha^{-1} had percent increase of oil and crude protein contents over control were 8.0 and 23.1% and 10.5 and 28.3%, respectively (Fig. 2). Nano-S fertilized plants produced kernels rich in oil and crude proteins. This may be due to the role of S in oil and protein synthesis besides S containing amino acids. Similar results were also obtained by Qiang *et al.* (2008) [29] who have reported that protein content of wheat was increased significantly by slow or controlled release fertilizers and felted by nano-materials compared with NPK chemical fertilizers. It was effective to use slow or controlled release fertilizer coated by nano-materials to improve wheat quality parameters. Zeolite application as a soil conditioner improved the oil percentage and oil yield of sunflower under withholding at stem elongation and seed filling stage (Karimi *et al.*, 2013) [18].

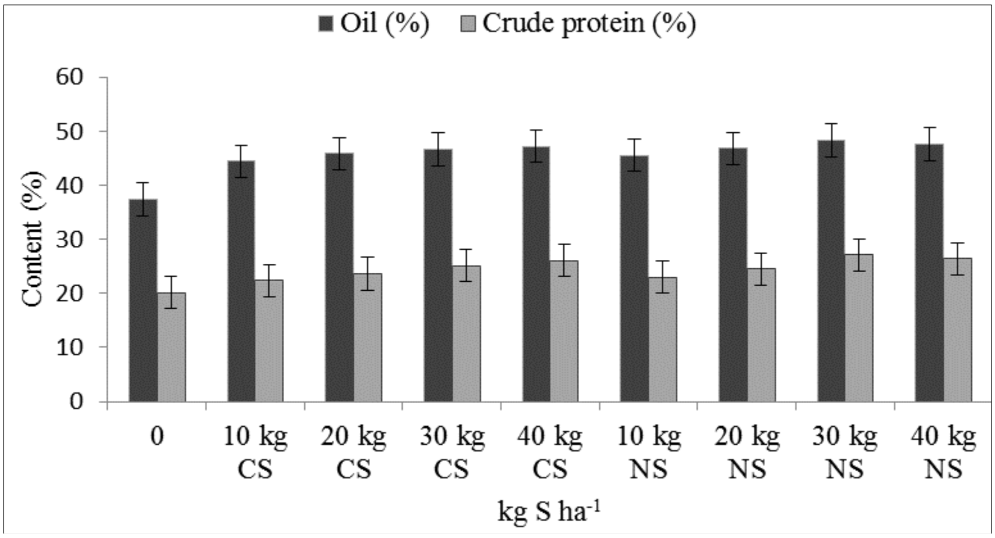

Fig 2: Effect of nano-sulphur on oil and crude protein content of groundnut

Sulphatase activity

The data showed that soil sulphatase activity increased remarkably at 30 DAS but its effects were less pronounced 60 DAS and harvest stage. Application of NS @ 30 kg S ha^{-1} recorded higher sulphatase activity of 39.4, 34.1 and 26.1µg p-nitrophenol g^{-1} of dry soil h^{-1} at 30, 60 DAS and post-harvest stage, respectively (Table 3). The results showed that the considerable increase in the soil sulphatase activity at 30 DAS due to application of sulphur fertilizers after that its activity was declined. Arylsulfatase activity can be affected by SO_4^{2-} concentration since available sulphur status increased in the experimental soil due to slow and steady release of sulphur from surface modified nano-zeolite based fertilizer during growth period (Knauff *et al*., 2003). Arylsulfatase is an enzyme that hydrolyzes organic S esters and releases SO_4^{2-} and hence plays an important role in organic S mineralization (Chen *et al*., 2001). Similar results were also registered by Selva Preetha (2011) [32] who has stated that the nanocomposites treatment substantially increases enzymes activities such as nitrate reductase, catalase and peroxidase activity.

Table 3: Effect of nano-sulphur on sulphur fractionation of post-harvest soil

S Levels (Kg S ha^{-1})	Sulphur fractionation (ppm)									
	H_2O soluble-S		Adsorbed-S		Occluded-S		Organic-S		Total-S	
	CS	NS	CS	NS	CS	NS	CS	NS	CS	NS
L_{10}	6.1	6.4	3.2	3.4	64.6	63.8	172.5	172.7	246.4	246.3
L_{20}	6.4	7.1	3.4	3.7	65.4	65.0	171.6	171.4	246.9	247.2
L_{30}	7.1	7.7	3.6	4.1	66.7	64.8	170.4	171.3	247.9	247.9
L_{40}	7.4	8.3	3.9	4.4	67.1	64.2	170.2	172.5	248.6	249.4
Control	3.5		1.7		61.2		172.1		238.5	
	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)	SEd	CD (P=0.05)
L	0.13	0.28	0.07	0.15	1.23	NS	3.23	NS	4.66	NS
F	0.09	0.20	0.05	0.10	0.87	NS	2.28	NS	3.29	NS
LxF	0.18	0.39	0.10	0.21	1.74	NS	4.57	NS	6.58	NS

Sulphur use efficiency (SUE)

Application of nano-S fertilization @ 30 kg S ha^{-1}recorded highest SUE of 29.6%. Whereas the conventional S fertilized plants at same dose was found lowest SUE of 16.4%. (Fig. 3). This might be due to slow and steady release mechanism of surface modified nano-zeolite based fertilizer besides reducing the various losses such as leaching, fixation and oxidation losses of sulphur. These findings are similar with results of Li and Zhang (2010) [22] who have stated that compared to water soluble sulfate, the sulfate sorbed on SMZ could be slowly released. Subramanian and Tarafdar (2011) [34] revealed that ^{15}N studies were taken using maize as a model system have revealed that N use efficiency from nano-fertilizer was 82 per cent and the conventional fertilizer (urea) registered 42 per cent with a net higher nitrogen use efficiency of 40 per cent which is hardly achievable in the conventional systems.

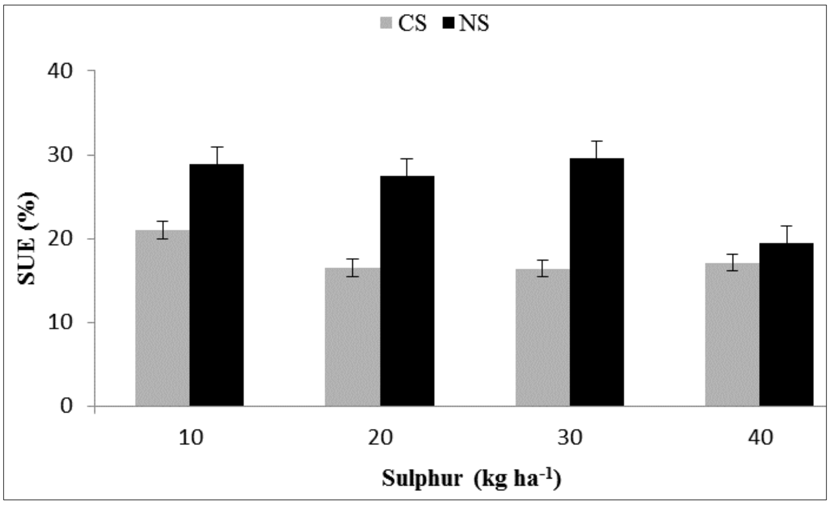

Fig 3: Effect of nano-sulphur on sulphur use efficiency of groundnut

Nano-S and conventional Son sulphur fractionation pattern of the soil

The application of NS @ 40 kg S ha^{-1} registered highest water soluble S (8.3 ppm), exchangeable S (4.4 ppm) and total sulphur S (249.4 ppm). Whereas CS fertilized soil at same dose recorded lower water soluble S (7.4 ppm), exchangeable S (3.9 ppm) and total S (248.6), in comparison to NS fertilized soil. The control recorded lowest water soluble S fraction of 3.5 ppm (Table 4). The higher soil occluded S fraction of 67.1 ppm was recorded in CS fertilized @ 40 kg S ha^{-1}. The NS applied soil at similar S dose registered lowest occluded S fraction of 64.2 ppm, in comparison to CS fertilized treatment. The higher organic S fraction (172.7 ppm) was observed in NS @ 10 kg S ha^{-1} fertilized soil, which was statistically comparable with CS applied at same dose of S (172.5 ppm). There was no much variation in the organic S fraction among the CS and NS treatments.

The water soluble and adsorbed sulphur fraction was increased in the NS received soil and it is mainly due to slow and steady release behaviour of the nano-zeolite based fertilizer. Whereas occluded sulphur was registered the highest in the sulphur applied as conventional fertilizer might be due to conventional fertilizer are readily available in nature thus it was fixed in the clay minerals in the soil while sulphur applied as surface modified nano-zeolite it was slowly soluble thereby slow release of nutrients takes place. These results are close agreement with Balanagoudar and Satyanarayana (1990) [2] who have reported that occluded sulphur or non sulphate sulphur it is mostly 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 sulphate sulphur.

The total sulphur content of the soil increased with an increase in organic carbon and finer fraction of the soil. Similar results were also reported by Basumatary *et al.* (2008) [3]. The organic sulphur was the dominant form of fraction in the soil. The variation in the organic sulphur is mainly due to mineralization and oxidation of this sulphur and also by varied based on organic carbon content and finer fraction of the soil. These findings corroborate the results of Jat and Yadav (2006) [14]. Adsorbed sulphur accounted for the smallest fraction of the total sulphur and this sulphur was extracted from exchange complex of the soil by the extractant. Organic carbon, soil pH and CEC content of the SMNZ applied soil had registered highest values thus increased the variation in the adsorbed sulphate content. Gowrisankar and Shukla (1999) [9] also reported similar observation.

Overall, the experimental results indicated that root, shoot, kernel and shell sulphur uptake, amino acids, pod and haulm yield, oil content, crude protein, sulphatase activity, various sulphur fractions and available sulphur status were significantly influenced with application of nano-S @ 30 kg S ha^{-1}, in most cases it was comparable to nano- S and conventional S @ 40 kg S ha^{-1}. The finding concluded that nano-sulphur is potential sulphur fertilizer to attain higher sulphur use efficiency with a reduction in sulphur fertilization by 25% and also build up soil sulphur reserve without harming the soil environments.

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