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Characterization and Assessment of Synthesized Nano Sulphur in Combination with Organic Amendment on the Availability of Sulphur in Calcareous Soil

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ABSTRACT

Background: Sulphur is rapidly being recognized as fourth key nutrient for plants after nitrogen, phosphorus and potassium. It functions in several critical metabolic and physiological processes, such as Chlorophyll synthesis, Protein synthesis, Activation of enzymes, Stress tolerance and Seed production. This study was carried to investigate the effect of synthesized nano sulphur in combination with organic amendment to study available sulphur status in calcareous soil.

Methods: Nano sulphur was synthesized using reverse microemulsion (water-in-oil microemulsion) technique and characterized at the Centre for Agricultural Nanotechnology, TNAU, Coimbatore. An incubation experiment was carried out with synthesized nano sulphur and gypsum as sulphur source with and without FYM to determine sulphur release at various intervals (7^{th}, 15^{th}, 30^{th}, 45^{th} and 60^{th} days after incubation).

Result: It was observed that nano sulphur applied at 40 kg S ha^{-1} with FYM showed high release of sulphur in calcareous soil in all intervals analysed during the experiment. The addition of organic manures aids in proliferation of heterotrophic sulphur oxidising bacteria which enhances the sulphur oxidation in alkaline soil.

Key words: Available sulphur, Calcareous soil, Microemulsion, Nano sulphur.

INTRODUCTION

Sulphur is rapidly being recognized as fourth key nutrient for plants after nitrogen, phosphorus and potassium (Rai *et al*., 2020). It functions in several critical metabolic and physiological processes, such as Chlorophyll synthesis (Chung *et al*., 2022), Production of proteins (Kopriva *et al*., 2019), Enzyme stimulation (Asghari *et al*., 2020), Stress tolerance (Mangal *et al*., 2023), Production of seeds (Zenda *et al*., 2021).

Calcareous soils are widespread in the semi-arid and arid regions (FAO, 2016), covering more than 30% of planet's surface and range in $CaCO_3$ content from hardly detectable to 95% (Taalab *et al*., 2019). A total of 38 out of 60 agro-ecological subregions in India have calcareous soils, which are projected to cover 228.8 million hectares (ha) or 69.4% of the nation's total geographical area (Pal *et al*., 2000). These soils lack many nutrients, hence proper nutrient management practises are needed to close the gap between current and maximum attainable yields (Wahba *et al*., 2019) (Gaytán Martínez *et al*., 2022) (Alghamdi *et al*., 2023). Sulphur insufficiency has been identified as major shortfall that contributes to low crop production of these soils (Khalid *et al*., 2012). In calcareous soils, pH alteration has been used to increase the accessibility of nutrients that are pH-sensitive. Sulphur is the most common and cost-effective acidifier. To lower the pH of calcareous soil and thereby boost crop nutrient availability, elemental sulphur (ES) application has been suggested (Amin and Mihoub, 2021) (Wahba *et al*., 2019). To use elemental sulphur as a fertiliser effectively and get best crop yields, it is vital to understand sulphur oxidation and how it is distributed in calcareous soils (Singh *et al*., 2014). In alkaline calcareous soil, biochemical oxidation of ES results in production of sulphuric acid, which lowers the pH of soil and dissolves $CaCO_3$ (Akila *et al*., 2022) (Besharati, 2017).

In sulphur insufficient soils, elemental form of sulphur (S°) is ineffective, instead it must be first transformed into sulphate sulphur by soil bacteria such as sulphur oxidising bacteria. S° is typically converted to sulphate faster when it is in smaller particle sizes and mixed with a substrate or soil (Shakoor *et al*., 2023). By incorporating organic amendments, sulphur oxidation and mineralization processes worked together to increase the amount of sulphur that was available

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to plants from elemental sulphur, a slow-releasing S source (Malik *et al*., 2021). Nano sized sulphur will be a better option for overcoming the delay in transformation of elemental sulphur into sulphate sulphur. Moreover, addition of organic manure to sulphur improves the effectiveness of organic manure and its impact on chemical characteristics of calcareous soil (Elbaalawy and Abou Hussien, 2020).

Nanotechnology has lot of potential as a growing area of agricultural research. Due to their great performance, low environmental dangers and low cost, nano fertilisers may be superior to their inorganic counterparts (Tipu *et al*., 2021).

Nanoparticles and their composites can be applied directly to plants as fertilisers and fungicides, or they can be used more delicately in diagnostics and precision agriculture (Elmer *et al*., 2018). Nano sulphur was compared with conventional sulphur in response to groundnut and it was observed that 30 kg ha^{-1} of nano sulphur was on par with 40 kg ha^{-1} of conventional sulphur in recording the highest pod yield of groundnut (Thirunavukkarasu *et al*., 2018).

In this background nano sulphur was synthesized by reverse micro emulsion technique and there have been limited studies regarding the interactive effect of elemental sulphur and organic manure on sulphur availability, pH and EC of calcareous soils. Hence this present study was conducted with the objectives (i) to synthesize nano sized sulphur fertilizers (ii) to evaluate the interactive effect of organic manure and nano sulphur on pH, EC and available sulphur in calcareous soil.

MATERIALS AND METHODS

Synthesis of sulphur nano particles

Synthesis of nano sulphur involves stirring and mixing of two reverse microemulsions. In a reverse microemulsion, the water phase is dispersed as tiny droplets in oil phase instead of being the continuous phase like in a regular emulsion, where the oil is the dispersed phase and the water is the continuous phase.

Reverse microemulsions can also be used as templates for synthesis of nanoparticles, where the dispersed phase contains the precursor chemicals for the desired nanoparticles. The small size and high stability of the emulsion droplets can allow for precise control over the size and properties of the resulting nanoparticles. Reverse microemulsion's "tiny pool" can be used as a "micro reactor" for synthesising nanoparticles.

Stable reverse microemulsions were obtained by mixing cyclohexane as oil phase, ethanol as co-surfactant, the non-ionic surfactant tween-80 and 6 mol/L sodium polysulfide solution (microemulsion I) or 6 mol/L hydrochloric acid solution (microemulsion II) as aqueous phases. Cyclohexane, tween-80 and ethanol were mixed under continuous stirring till the blend turn out to be transparent. Then appropriate amount of 6 mol/L sodium polysulfide solution (microemulsion I) or 6 mol/L hydrochloric acid solution (microemulsion II) was supplemented dropwise under strong stirring till the blend turn out to be transparent. The microemulsion II was added dropwise to microemulsion I under stirring, at room temperature. At the end of the reaction, acetone was combined to microemulsion to source precipitation of sulphur nanoparticles synthesized in microemulsion. The precipitate was removed by centrifugation and repeatedly washed with acetone, methanol and water to eliminate residual organic constituents and salt formed (NaCl) from the product to make it pure; then the product was dried in an oven (Fig 1).

Equation depicts the reaction that resulted from the blending of these two microemulsions.

$$Na_2S_x + 2HCl \rightarrow 2NaCl + H_2S + (x\text{-}1)\ S.$$

Characterization of nano sulphur

The Nanopartica SZ-100, manufactured by Horiba Scientific in Japan, was used to measure particle size. Particle size

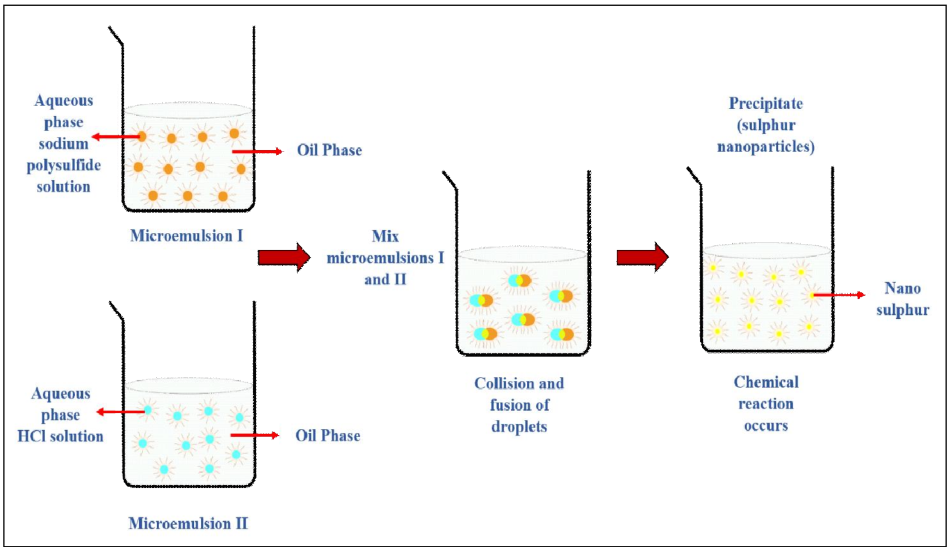

Fig 1: Synthesis of sulphur nano particles by microemulsion technique.

analyzer measurements were taken after accurately dispersing 1.0 mg of the nano sulphur in 20 mL of dispersion media after ultrasonication. The zeta potential was also measured concurrently by means of same apparatus in which zeta potential electrostatic forces were measured between -200 mv and +200 mv in order to evaluate the stability of particles.

UV-VIS spectroscopy was executed using SPECORD 210 PLUS model with spectral scan as measurement mode at a speed of 20 nm/s. Using TEM (Quanta 240, FEI Techni Sprit, Netherlands), the powdered sample was photographed.

Incubation experiment

An incubation experiment was carried out with synthesized nano sulphur and gypsum to study the release of sulphur. The soil taken for the study was sandy clay loam texture, calcareous sulphur deficient soil (9.7 mg kg^{-1}). The soil reaction was alkaline with pH-8.5, EC- 0.27 dSm^{-1}, free $CaCO_3$ content - 14%. The NPK status and organic carbon status was 150 kg ha^{-1}, 11 kg ha^{-1}, 255 kg ha^{-1} and 2.8 g kg^{-1} respectively. The experiment was conducted in a completely randomized block design with eight different treatments and three replications. The treatment details were given in Table 1. The initial characteristics of incubation soil is given in Table 2. The FYM was applied at the rate of 12.5 t ha^{-1}. 200 grams of 2 mm sieved soil was taken in plastic jar and the treatments were imposed. Each incubation jar was maintained at 60% of its water holding capacity throughout the experiment. The available sulphur was analysed by taking 5 grams of soil extracting with 0.15% $CaCl_2$. To 5 mL of the extract, 10 mL of sodium acetate and acetic acid buffer and 1 mL of gum acacia was added. 1 g of $BaCl_2$ was added at the time of absorbance reading and the solution was made up to 25 mL. The absorbance of the solution was read at 420nm (Chesnin and Yien, 1951).

Data acquisition

Soil samples were collected treatment-wise from the incubation jar at periodic intervals of (7th, 15th, 30th, 45th and 60th days after incubation) processed and analysed for pH, EC and available sulphur.

Statistical analysis

The statistical analysis for experimental data was performed separately for each interval of incubation by using SPSS software (version 16.0). One-way ANOVA (analysis of variance) was done for all the parameters and the results were presented as mean with standard error. The mean values of eight treatments were ranked by using Duncan's multiple range test (DMRT) at p = 0.05.

RESULTS AND DISCUSSION

Particle size analysis and zeta potential

The nano sulphur particles synthesized from microemulsion technique was analysed for its size and stability using particle size analyser with scattering angle 90° and temperature was maintained at 25°C and result was represented as scattering light intensity. The polydispersity index was 0.080 and Z-average is 177.5 nm (Fig 2). This indicates nano sulphur is monodispersed with consistent size, shape and mass distribution. The zeta potential was measured in same instrument and observed as -45.6 mV, verifying long term colloidal stability (Fig 3) (Subramanian *et al*., 2022).

UV-VIS Spectroscopy

The synthesized nano sulphur was characterized using UV-VIS spectroscopy by their strong ultra violet absorption. The absorption was measured in SPECORD 210 PLUS model

Table 1: Treatment details of the incubation experiment.

T1	:	Control
T2	:	Gypsum (S 40 kg ha^{-1})
T3	:	Nano sulphur (20 kg ha^{-1})
T4	:	Nano sulphur (40 kg ha^{-1})
T5	:	Control + FYM
T6	:	Gypsum (S 40 kg ha^{-1}) + FYM
T7	:	Nano sulphur (20 kg ha^{-1}) + FYM
T8	:	Nano sulphur (40 kg ha^{-1}) + FYM

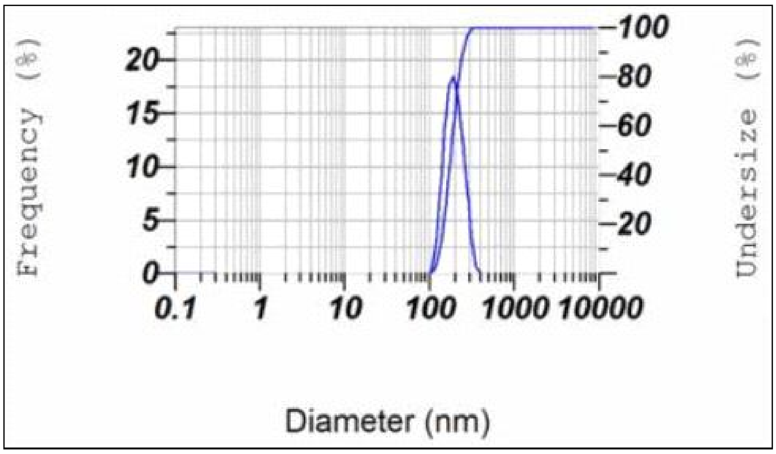

Fig 2: Particle size analysis of nano sulphur.

as a function of wavelength with the spectral scan as measurement mode at a speed of 20 nm/s. It collects spectra from 190 - 1100 nm using a slit width of 1 nm. The maximum absorption spectra band was visible at 277 nm which is indicative of perfect sulphur nano particles (Fig 4) (Huergo *et al*., 2019) (Khan, 2012) (Suryavanshi *et al*., 2017).

Tem analysis

TEM was used to analyse nano structured materials with atomic scale resolution. TEM image revealed average particle size between 16.5 nm-25 nm (Fig 5). The homogenous nature of nano sulphur was supported by its particle distribution and surface morphology.

Incubation experiment

The pH of the soil analysed at different intervals are presented in the Table 3. The application of sulphur at different sources and forms altered the pH of experimental soil. Soil pH was observed to decrease throughout the incubation experiment from 8.35, 8.27, 8.17, 8.09, 7.99 at 7, 15, 30, 45 and 60 DAI, respectively. Among the different doses of sulphur nano sulphur at 40 kg ha^{-1} along with FYM (from 8.10 to 7.45) showed the maximum decrease in soil pH, followed by nano sulphur at 20 kg ha^{-1} +FYM (from 8.19 to 7.72) and gypsum at 40 kg ha^{-1} +FYM (from 8.27 to 8.10). Regardless of FYM applied, a constant decrease in soil pH

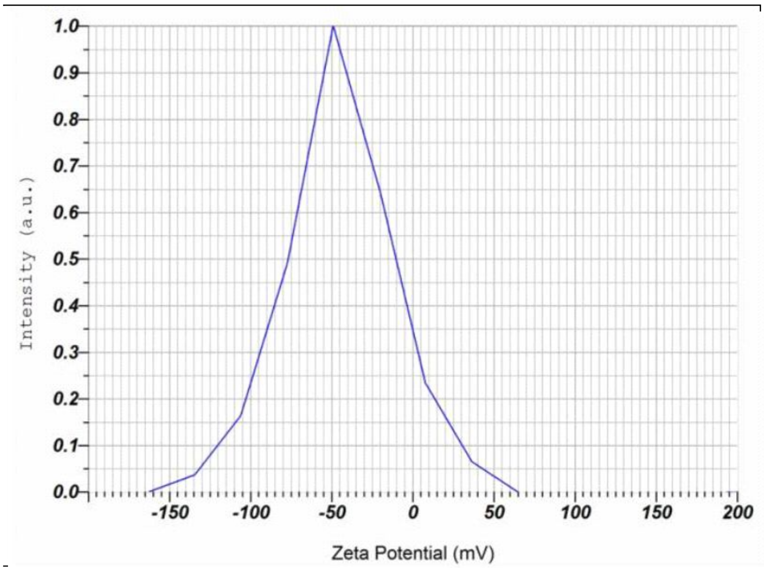

Fig 3: Stability analysis of nano sulphur.

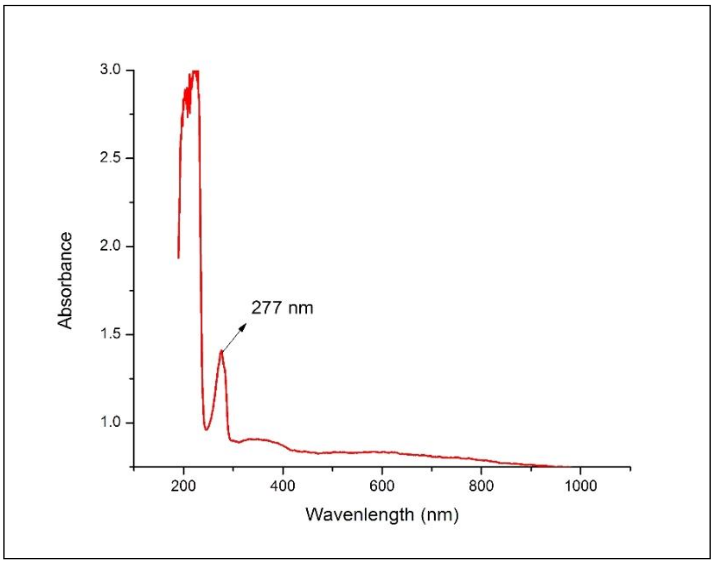

Fig 4: Prominent peak at 277 nm is visible in the UV-VIS spectra of nano sulphur.

was observed with increasing sulphur levels at each periodic interval of incubation. The absolute decrease in pH could be attributed to higher level of S fertilization, which had a significant acidulating effect. Low soil pH levels promote the quickest sulphate oxidation (Havlin *et al*., 2016). The principal result of applying sulphur to soil, especially calcareous soils, is to decrease soil pH caused by subsequent S oxidation. The efficiency of lowering the pH by sulphur oxidation is primarily influenced by the sulphur dose and the soil's ability to act as a buffer (Janzen and Bettany, 1987) (Slaton *et al*., 2001). Elemental sulphur, the standard acidulent used to lower soil pH, occurs in a reduced state and must be oxidised to sulphuric acid during the biochemical oxidation, which reduces soil pH. Additionally, nitrification releases hydrogen (H+) ions, which promote soil acidification and the accumulation of CO_2 during microbial respiration is most likely what causes the pH to fall. This might be reason for declined pH in the FYM amended treatments due to the presence of micro-organisms and organic acids (Anandham *et al*., 2010).

The EC of the soil analysed at different intervals was presented in the Table 4. The application of sulphur at different sources and forms altered the EC of experimental soil. Soil EC was observed to increase throughout the incubation from 0.28, 0.30, 0.34, 0.36 and 0.30 at 7, 15, 30, 45 and 60 DAI, respectively. Among the different doses of sulphur nano sulphur at 40 kg ha^{-1}+ FYM showed the maximum increase in EC up to 45 DAI and declined in 60 DAI, the same trend was followed by nano sulphur at 20 kg ha^{-1} +FYM and gypsum at 40 kg ha^{-1} +FYM. Regardless of FYM applied, a constant increase in soil EC was observed with increasing sulphur levels at each periodic interval of incubation up to 45 DAI.

The dosage of 40 kg S ha^{-1}+FYM caused the SO_4^{2-} concentration to raise in the soil due to sulphur oxidation. The sulphate present in soil due to oxidation combines with the Ca_2^+ in calcareous soil and forms soluble salts which caused the increase in EC (Soaud *et al*., 2011).

The available sulphur of soil analysed at different intervals was presented in Table 5. Different sources of sulphur, with and without FYM amendment had impact on sulphur release in calcareous soils. The observed data revealed gradual raise in sulphur release in calcareous soils during incubation period up to 45 DAI and a slight decline at 60 DAI. The mean increase in soil available sulphur varied from 10.23, 10.87, 11.29, 11.82 and 11.03 mg/kg at 7, 15, 30, 45 and 60 DAI respectively. In the incubation period, the highest availability of sulphur was recorded in T_8 - S as Nano sulphur at 40 kg ha^{-1} + FYM followed by T_7 - S as nano sulphur at 20 kg ha^{-1} +FYM and T_6 -S as gypsum at 40 kg S ha^{-1} + FYM.

Due to the lack of reduced sulphur compounds, thiobacillus spp., which have long been regarded as the sulpuhr oxidizers, are not usually found in considerable numbers in most sulphur deficient agricultural soils. Consequently, the significance of heterotrophic sulphur-oxidizing bacteria in this environment increases (Lawrence and Germida, 1991). In general, the growth and proliferation of heterotrophic bacteria depends on energy provided by organic carbon in soil. The addition of organic sources to the soil would have increased the diversity and metabolism of heterotrophic microbial population which might have accelerated sulphur oxidation (Germida and Janzen, 1993)

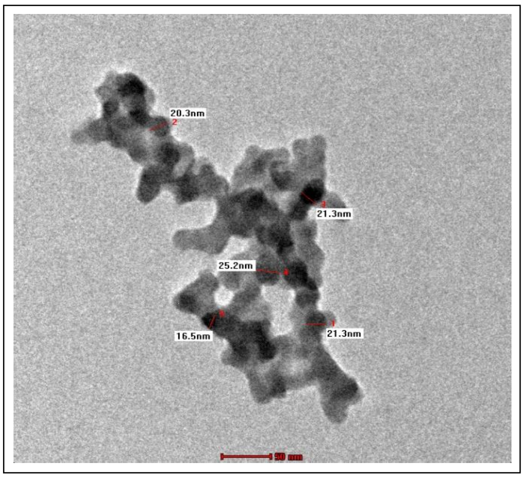

Fig 5: TEM images of nano sulphur.

Table 2: Physico-chemical properties of initial soil incubation experiment.

Physical properties	Eastern block, TNAU, Coimbatore
Bulk density (Mg m^3)	1.25
Particle density (Mg m^3)	2.07
Porosity (%)	6.59
Mechanical analysis	
Coarse sand (%)	29.7
Fine sand (%)	28.9
Silt (%)	15.3
Clay (%)	25.5
Textural class	Sandy clay loam
Electrochemical properties	
pH	8.5
EC (dS m^{-1})	0.27
CEC (c mol (p+) kg^{-1})	34.9
Chemical properties	
Organic carbon (g C kg^{-1})	2.8
Available Nitrogen (kg ha^{-1})	150
Available Phosphorus (kg ha^{-1})	11
Available Potassium (kg ha^{-1})	255
Available Sulphur (mg kg^{-1})	9.7
DTPA extractable Fe (mg kg^{-1})	5.29
DTPA extractable Zn (mg kg^{-1})	1.6
DTPA extractable Mn (mg kg^{-1})	2.9
DTPA extractable Cu (mg kg^{-1})	1.15
Free $CaCO_3$ (%)	14

(Pepper and Miller, 1978). The presence of high levels of labile carbon and nitrogen in the organic sources might have supplied the superior source of energy for the metabolic processes of chemoheterotrophic sulphur oxidizers during the early phase of incubation. The recalcitrant carbon present in the organic substrate which remained accessible to the proliferation of chemolithotrophs for a longer period might have caused a significant increase in the sulphur oxidation during later stages of incubation (Malik *et al*., 2021). The heterotrophic sulphur oxidation is enhanced by

Table 3: Effect of nano sulphur and nano sulphur combined with FYM on pH status of calcareous soils at different intervals.

Treatments	7 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T_1- Control	8.65±0.07 [a]	8.57±0.09 [a]	8.49±0.07[a]	8.46±0.22[a]	8.42±0.12[a]
T_2- Gypsum (S 40 kg ha^{-1})	8.47±0.17[ab]	8.39±0.08[ab]	8.20±0.11[ab]	8.16±0.19[abc]	8.11±0.01[ab]
T_3- Nano S (S 20 $kgha^{-1}$)	8.38±0.03[ab]	8.34±0.05 [ab]	8.24±0.12 [abc]	7.95±0.14 [bc]	7.91±0.19[b]
T_4- Nano S (S 40 $kgha^{-1}$)	8.27±0.16[ab]	8.16±0.04 [ab]	7.90±0.09 [abc]	7.89±0.18 [c]	7.87±0.19[b]
T_5- Control + FYM	8.46±0.10[ab]	8.43±0.05[ab]	8.45±0.22 [abc]	8.39±0.08 [ab]	8.37±0.01[a]
T_6- Gypsum (S 40 kg ha^{-1}) + FYM	8.27±0.09[ab]	8.21±0.01[ab]	8.16±0.01[bc]	8.12±0.11 [abc]	8.10±0.06 [ab]
T_7- Nano S (S 20 $kgha^{-1}$) + FYM	8.19±0.18[b]	8.11±0.02 [ab]	8.05±0.13[c]	7.92±0.12[bc]	7.72±0.12[bc]
T_8- Nano S (S 40 $kgha^{-1}$) + FYM	8.10±0.07[b]	7.94±0.07[b]	7.85±0.18[c]	7.83±0.05[c]	7.45±0.10[c]
Mean	8.35	8.27	8.17	8.09	7.99
SEd	0.17	0.21	0.18	0.21	0.17
CD (p<0.05)	0.36	0.44	0.39	0.44	0.36

Values are mean of three replicates ± standard error (n=3); values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p≤0.05).

Table 4: Effect of nano sulphur and nano sulphur combined with FYM on electrical conductivity of calcareous soils at different intervals.

Treatments	7 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T_1- Control	0.21±0.001 [f]	0.22±0.004[f]	0.23±0.006 [f]	0.22±0.001[e]	0.22±0.001[e]
T_2- Gypsum (S 40 kg ha^{-1})	0.25±0.002 [d]	0.27±0.003[d]	0.36±0.001[c]	0.42±0.004[c]	0.28±0.007[a]
T_3- Nano S (S 20 kg ha^{-1})	0.28±0.004 [c]	0.34±0.002 [b]	0.35±0.002[cd]	0.36±0.008[d]	0.32±0.001[b]
T_4- Nano S (S 40 kg ha^{-1})	0.32±0.006 [b]	0.34±0.003[b]	0.42±0.006[b]	0.45±0.010[b]	0.34±0.007[a]
T_5- Control + FYM	0.23±0.001 [e]	0.24±0.001[e]	0.24±0.001[f]	0.23±0.003[e]	0.24±0.005[d]
T_6- Gypsum (S 40 kg ha^{-1}) + FYM	0.27±0.004 [c]	0.30±0.005 [c]	0.33±0.002[e]	0.35±0.007[d]	0.32±0.005[c]
T_7- Nano S (S 20 kg ha^{-1}) + FYM	0.31±0.007 [b]	0.33±0.005 [b]	0.34±0.008[de]	0.35±0.001[d]	0.35±0.001[b]
T_8- Nano S (S 40 kg ha^{-1}) + FYM	0.34±0.006 [a]	0.38±0.008 [a]	0.45±0.007[a]	0.49±0.009[a]	0.35±0.004[a]
Mean	0.28	0.30	0.34	0.36	0.30
SEd	0.0065	0.0063	0.0063	0.0088	0.0063
CD (p<0.05)	0.013	0.013	0.0135	0.0187	0.0134

Values are mean of three replicates ± standard error (n=3); values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p≤0.05).

Table 5: Effect of nano sulphur and nano sulphur combined with FYM on available sulphur status of calcareous soils at different intervals.

Treatments	7 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T_1- Control	9.70±0.04[c]	9.71±0.18[d]	9.73±0.01[e]	9.81[e]±0.24	9.75±0.03[e]
T_2- Gypsum (S 40 kg ha^{-1})	9.81±0.05[c]	10.40±0.02[c]	10.24±0.19[de]	11.49[d]±0.13	10.18±0.04[de]
T_3- Nano S(S 20 kg ha^{-1})	9.97±0.22[c]	10.70±0.18[c]	10.64±0.09[d]	11.98±0.10[c]	10.63±0.07[cd]
T_4- Nano S(S 40 kg ha^{-1})	10.53±0.14[b]	11.43±0.29[b]	12.35±0.12[c]	12.58±0.25[b]	12.04±0.22[b]
T_5- Control + FYM	9.80±0.06[c]	9.82±0.20[d]	9.81±0.21[e]	9.83±0.10 [e]	9.79±0.12[e]
T_6- Gypsum (S 40 kg ha^{-1}) + FYM	9.99±0.17[c]	10.43±0.12[c]	10.71±0.26[d]	11.22±0.03[d]	11.02±0.25[c]
T_7- Nano S (S 20 kg ha^{-1}) + FYM	10.75±0.03[b]	11.87±0.06[b]	12.92±0.10[b]	13.67±0.01[a]	11.98±0.31[b]
T_8- Nano S (S 40 $kgha^{-1}$) + FYM	11.32±0.26[a]	12.57±0.20[a]	13.89±0.07[a]	13.99±0.16[a]	12.87±0.03[a]
Mean	10.23	10.87	11.29	11.82	11.03
SEd	0.21	0.25	0.22	0.21	0.24
CD (p<0.05)	0.44**	0.53**	0.46**	0.45**	0.50**

Values are mean of three replicates ± standard error (n=3); values followed by the same letter in each column are not significantly different from each other as determined by DMRT (p≤0.05).

the alkaline soil conditions. There are many studies revealing the high rate of sulphur oxidation alkaline soils or in response to the addition of $CaCO_3$ (Yang *et al*., 2007) (Czaban and Kobus, 2000).

CONCLUSION

Nano sulphur was successfully produced using the microemulsion method. The particle size analyser observed the polydispersity index as 0.080 and z-average as 177.5 nm. The zeta potential was recorded to be -45.6 mV confirming the long-term stability. The UV-VIS spectroscopy recorded the maximum absorption at 277 nm indicating the perfect sulphur nano particles. Nano sulphur was photographed using Transmission electron microscope and size was recorded between 16.5 nm -25 nm. From the incubation experiment, it was observed that nano sulphur applied at 40 kg S ha^{-1} combined with FYM showed the high release of sulphur in calcareous soil in all the intervals analysed during the incubation experiment. The addition of organic manures aids in proliferation of heterotrophic sulphur oxidising bacteria which enhances the sulphur oxidation in alkaline soil.

Conflict of interest: None.

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