Effect of Different Sulfur Levels from Various Sources on *Brassica napus* Growth and Soil Sulfur Fractions

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Summary: A two year field study was conducted at two different locations in northern rainfed Punjab, Pakistan to assess the effect of different rates of sulfur application from various sources on soil sulfur fractions and growth of *Brassica napus*. The treatments included three sulfur sources *i. e.*, single super phosphate, ammonium sulfate and gypsum each applied at five different rates (0, 10, 20, 30 and 40 kg S ha^{-1}). Sulfur application had a significant positive effect on the growth and yield parameters of *Brassica napus*. Among the sulfur sources ammonium sulfate resulted in maximum increase in plant growth and yield parameters, followed by single super phosphate. Sulfur content and uptake by crop plants was significantly higher with ammonium sulfate application as compared to other two sulfur sources. Sulfur application also exerted a significant positive effect on different S fractions in the soils. On an average, 18.0% of the applied sulfur got incorporated into $CaCl_2$-extractable sulfur fraction, while 15.6% and 35.5% entered into adsorbed and organic sulfur fractions in the soils, respectively. The value cost ratio increased significantly by sulfur application up to 30 kg ha^{-1}. Among sulfur sources, ammonium sulfate performed best giving the highest net return.

Introduction

Brassica napus belongs to family Cruciferae. It is commonly known as *gobi sarson* or *japani sarson*. It is an annual *Rabi* crop, 50-200 cm tall, having taproot system and is self pllonated. Fruit is pod, 5-10 cm long. Each pod contains 15-40 small seeds weighing 4-6 g per thousand seeds . It grows best in well drained light textured soils of temeperate regions [1]. In Pakistan, it is grown on an area of 194 thousand hectares with a production of 168 thousands tonnes [2].

Sulfur (S) is an essential plant nutrient and plays a vital role in the growth and development of crop plants. It is a component of amino acids cysteine and methionine, which act as precursor for the synthesis of all other compounds containing reduced S [3]. It also helps in chlorophyll synthesis, enhances the vegetative and reproductive growth in plants and increases oil contents in oil seeds [4, 5].

Intensification of agriculture with high yielding crop varieties and multiple cropping coupled with use of high analysis S-free fertilizers has accelerated the deficiency of S in soils in many countries of the world [6]. Situation is not different in Pakistan where there is rising trend of use of high grade S free fertilizers such as Urea and DAP.

Sulfur occurs in organic and inorganic forms and is cycled between these forms thorough various processes such as mobilization, mineralization, immobilization, oxidation and reduction [7]. Out of total sulfur, 95 percent is in organic and 5 percent is in inorganic form. Organic fraction is composed of carbon bonded (50 percent), non carbon bonded (40 percent) and soil biomass S (10 percent). Carbon bonded S is further subdivided into amino acid bonded (25 percent) and lipid bonded (25 percent) sulfur. Inorganic fraction is comprised of liquid phase and solid phase S. Liquid S exists in the form of sulfate and non sulfate while solid phase is found as adsorbed on clay, soil solids and Fe/Al oxides [7].

Opinion is divided regarding effect of inorganic S application on different S fractions in soil according to the results of trials conducted at Rothamsted [8, 9], in Norway [10] and in New Zealand [11, 12]. Further more, information regarding comparative effect of different S sources on soil S fractions is lacking. Therefore present study was conducted to, 1) evaluate the growth and yield response of *Brassica napus* to different application rates and sources of S 2) to evaluate the effect of different rates and sources of S applied on S fractions in soil, and 3) to analyze the economics of the use of S fertilizers for *Brassica napus* production under rain-fed dry farming.

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Results and Discussion

Response of Brassica napus to Sulfur

Brassica napus responded positively to S application at both the experimental sites in the study (Table-1). The mean increases in salique plant^{-1}, biomass yield and seed yield were 2.4 to 11.2 %, 0.4 to 1.7 %, and 1.0 to 4.7 % over the control, respectively with S application of 10 to 40 kg ha^{-1}. There was 4.4 to 9.6 % increase in oil concentrations with S application of 10 to 40 kg ha^{-1}, respectively. Significantly higher mean salique plant^{-1}, biomass yield and seed yield were obtained at the Rawal (*Inceptisols*) soil than at the Therpal (*Alfisols*) soil (Table-1). Similarly S uptake by crop plants was also significantly higher at the Rawal than at the Therpal soil. It was also evident that crop response to S fertilization was better during first year (2004-05) of the study at both the experimental sites (Table-1).

Rate of Sulfur Application

Although the positive effects of S on different plant growth and yield parameters *i. e.*, salique plant^{-1}, biomass yield, and seed yield were evident at all the levels of applied S, however a significant increase in above indices occurred at S application rate of 20 kg ha^{-1} and above (Table-1). On the other hand, a significant increase in oil concentrations was evident even at the lowest rate (10 kg ha^{-1}) of S application. Among the different rates of S tested in this study, the 40 kg S ha^{-1} produced higher mean salique plant^{-1}(318), biomass yield (9058 kg ha^{-1}) and seed yield (1656 kg ha^{-1}) but these values were statistically at par with 30 kg S ha^{-1}. The maximum mean oil concentration (44.6%) was recorded with 30 kg S ha^{-1}, which was also statistically similar to those obtained with 40 kg S ha^{-1}. The S concentrations and S uptake by *Brassica napus* plants also increased significantly (11.7 to 73.3%, and 15.0 to 80.0%, respectively) with increasing rates (10 to 40 kg S ha^{-1}) of S application. At the S application rates of 30 and 40 S kg ha^{-1}, the uptakes of S by *Brassica napus* plants were statistically similar to each other. This could be one of the reasons for similarity in the growth and yield response of *Brassica napus* crop at these application rates (30 and 40 kg S ha^{-1}). On the other side, S uptake by crop plants as percentage of the added S decreased drastically at S application rate of 40 kg S ha^{-1} (Fig. 1a).

Sources of Sulfur

Among different S fertilizers, stimulation of crop growth and yield parameters was highest in plots receiving ammonium sulfate (AS), followed by single super phosphate (SSP) and gypsum (G), but differences were not significant (Table-1). This indicated effectiveness of all the three S sources for improving S nutrition of *Brassica napus* crop under the agro-climatic conditions of this study. However, there were significant differences regarding plant S concentrations and S uptake by crop plants in response to the applied S sources. The AS resulted in significantly higher S contents and S uptake by *Brassica napus* plants than G (Fig. 2a).

Table-1: Main effects of S application rate (kg ha^{-1}), sources of S, soil depth, location and year on different S fractions in soil in two year field experiment.

Treatments	$CaCl_2$-S (μg g^{-1} soil)	Adsorbed S (μg g^{-1} soil)	Organic S (μg g^{-1} soil)	Total S (μg g^{-1} soil)
Rates of S (kg S ha^{-1})				
0	4.6 d	4.1 c	71.3 c	80.0 d
10	5.7 c	4.7 b	72. 6 bc	83.0 c
20	6.4 b	5.9 a	74.4 b	86.7 b
30	6.6 a	6.2 a	77.0 a	89.8 a
40	6.8 a	6.4 a	77.6 a	90.8 a
LSD value	0.35**	0.62*	2.9*	2.8*
Sources of S				
SSP	6.3 a	5.1 a	76.2	87.6 ab
AS	5.5 b	4.7 b	76.1	86.3 b
G	6.2 a	5.0 a	77.9	89.1 a
LSD value	0.10*	0.25*	NS	1.7*
Rates × Sources Interaction				
LSD value	NS	NS	NS	NS
Soil depth (cm)				
0-15	5.7 b**	5.5 a	78.4 a	89.6 a
15-30	6.5 a	4.6 b	72.3 b	83.4 b
Locations				
Rawal	5.6 b*	6.2 a	78.7 a	90.5 a
Therpal	6.5 a	5.3 b	74.5 b	86.3 b
Years				
2004-2005	4.8 b**	4.7 b	73.8 b	83.3 b
2005-2006	6.5 a	6.6 a	79.6 a	92.7 a

Means with different letters differ significantly according to Least Significant Difference (LSD) test (*P<0.05*). NS stands for non significant difference, SSP stands for single super phosphate, AS for ammonium sulfate and G for gypsum. * and ** denote significance at $P < 0.05$ and $P < 0.01$ levels, respectively.

Table-2: Physical and chemical characteristics of experimental soils.

Characteristics	Rawal (*Inceptisols*)		Therpal (*Alfisols*)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Sand (%)	45	43	69	66
Silt (%)	40	40	21	21
Clay (%)	15	17	10	13
EC_e (dS m^{-1})	0.38	0.43	0.30	0.35
pH	7.8	7.9	7.9	8.0
$CaCO_3$ (%)	11.3	12.2	5.2	5.6
Organic Carbon (mg g^{-1} soil)	2.90	2.82	2.78	2.70
Total N (mg g^{-1} soil)	0.30	0.27	0.28	0.20
Total S (μg g^{-1} soil)	93.7	87.7	86.1	75.8
Organic S (μg g^{-1} soil)	79.8	74.2	76.4	67.3
Adsorbed S (μg g^{-1} soil)	6.6	5.4	3.9	2.1
$CaCl_2$-S (μg g^{-1} soil)	7.3	8.1	5.8	6.4

Soil Sulfur Fractions

In the top layers (0-15 cm) of Rawal and Therpal soils used in this study, the soil solution S (0.01 $CaCl_2$-extractable S) accounted for 7.8% and 6.7% of the total S, respectively at the start of experiment (Table-3). The adsorbed S accounted for 7.0% and 4.5%, while the organic S accounted for 85.2% and 88.7% of the total soil S, respectively.

Sulfur application had a significant positive effect on different S fractions in the soils (Table-2). The mean concentrations of $CaCl_2$-extractable S, adsorbed S and total S increased significantly at all the rates of applied S, while a significant increase in the organic S occurred with S application at 20 kg ha^{-1} and above. There was 23.9 to 47.8% increase in $CaCl_2$-extractable S, 14.6 to 56.1% increase in adsorbed S, 1.8 to 10.9% increase in organic S and 3.7 to 15.4% increase in total S contents in the soils in response to S application from 10 to 40 kg ha^{-1}, respectively. On the average, 18.0% of the applied S got incorporated into $CaCl_2$-extractable S fraction, while 15.6% and 35.5% got incorporated into adsorbed S and organic S fractions in the soils, respectively (Fig. 1b-d). Among the S sources, SSP and G resulted in significantly higher increase in $CaCl_2$-extractable S, and adsorbed S fractions in the soils as compared to AS, while the organic S fraction did not show any significant difference with respect to applied S sources (Table-2). The contribution of AS to $CaCl_2$-extractable S and the adsorbed S fractions was lowest (8.0% and 5.4% of the added S, respectively) among the S sources (Fig. 2b-d). Almost similar fractions of the SSP and AS (44.0% and 43.1% of the added S, respectively) got incorporated into organic S pool, whereas the contribution of G to organic S pool was much greater (59.3% of the added S) than the other two, probably because of slow release of SO_4^{-2}-S from this source.

Fig 1 (a)

Fig 1 (b)

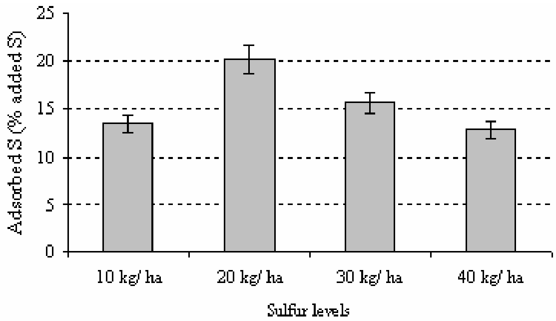


Fig 1 (c)

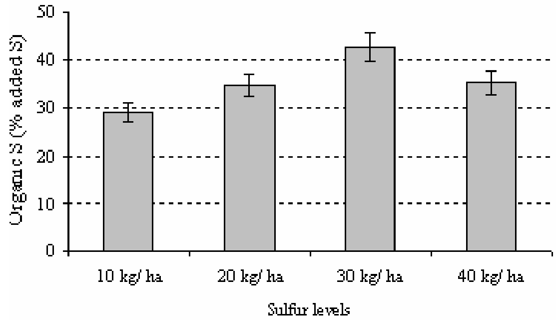


Fig 1 (d)

Fig. 1 Main effects of doses of sulfur (S) on uptake of S by *Brassica napus* crop and on incorporation into different S fractions in soil as % of the added S: (a) S uptake by *Brassica napus*; (b) 0.01M $CaCl_2$-extractable S; (c) adsorbed S and (d) organic S in a two year field study.

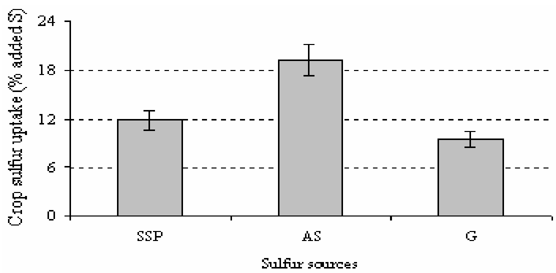
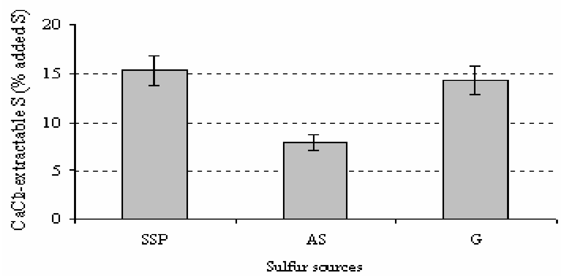


Fig 2 (a)



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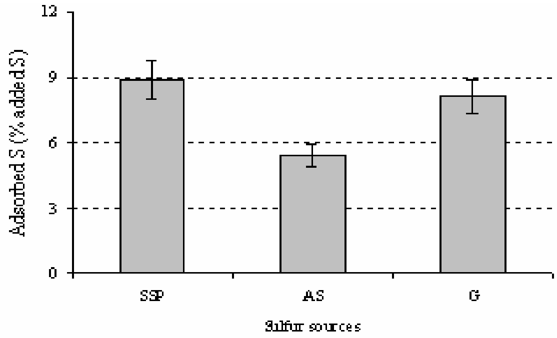

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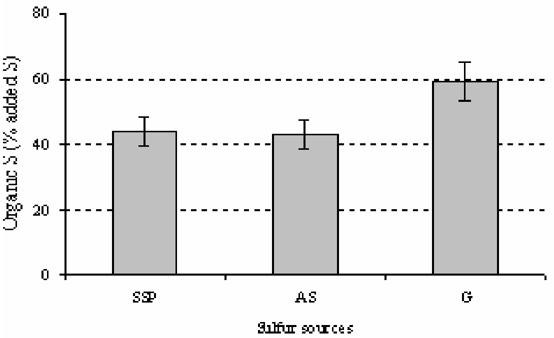

Fig 2 (d)

Fig. 2: Main effects of sources of sulfur (S) [single super phosphate (SSP), ammonium sulfate (AS), gypsum (G)] on uptake of S by Brassica napus crop and incorporation into different S fractions in soil as % of the added S: (a) S uptake by Brassica napus; (b) 0.01M $CaCl_2$-extractable S; (c) adsorbed S and (d) organic S in a two year field study

Economic Analysis

The application of all three S sources for the S nutrition of *Brassica napus* crop in this study was profitable, because the mean value cost ratio (VCR) values for the S sources were higher than 1.0 (Fig. 3a). The VCR values increased significantly by S application up to 30 kg S ha^{-1}, while at 40 kg S ha^{-1} declined drastically (Fig. 3a). Thus, the application of 30 kg S ha^{-1} proved to be the point of maximum return from the S fertilization in this study, and further increase in S application rate did not prove to be economical. Among the S sources, AS performed better, giving highest net return for each unit of applied S, and was followed by the SSP. However, there were negative marginal rate of return (MRR) values for AS at 40 kg S ha^{-1} application rate (Fig. 3b).

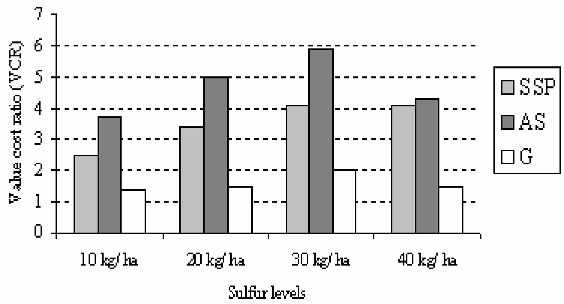

Fig 3 (a)

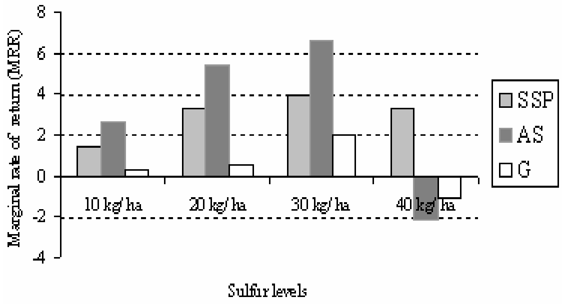

Fig 3 (b)

Fig. 3: Main effects of doses and sources of sulfur (S) on (a) value cost ratio (VCR) and (b) marginal rate of return (MRR) of *Brassica napus* crop in a two year field study

Response of Brassica napus to Sulfur

There was significant increase in seed yield and yield components of *Brassica napus* due to S application. In some previous studies, yield response of *Brassica napus* has been reported to vary from 10 to 80 % with application of 10 to 50 kg S ha^{-1} [13, 14]. In the present study however, the magnitude of yield response of *Brassica napus* to S fertilization was much lower than in the previous studies. The reasons for this apparently lower response in this study could be difference in agro-climatic conditions, and the cultivar types involved in the studies. The previous studies were mostly conducted in the areas with sufficient availability of water for crop plants in the form of irrigation water or rainfall for meeting crop water requirements. The present study was carried out in the area under rain-fed dry farming where moisture stress prevails during most part of the growing season due to insufficient winter rains, and is one of the main causes of low crop productivity in the area [15].

Seed and biomass yield and S uptake were significantly higher at the Rawal (*Inceptisols*) than at the Therpal (*Alfisols*) soil (Table-3). This could be attributed to variations in the rainfall and soil fertility at the experimental sites. Better moisture availability through rainfall and more clay contents at the Rawal soil might have contributed to better crop response to S fertilization at the Rawal soil. The role of soil properties particularly, the soil texture, and the rainfall in S availability to crop plants has been highlighted by many researchers [16, 17].

Crop response to S application was better during first year as compared to second year. This could be related to better environmental conditions throughout the crop growth period in the year 2004-05 as compared to 2005-06. Rainfall during the growing season of 2005-06 was almost half of that received during the same period in 2004-05. *Brassica napus* is a moisture sensitive crop whose growth decreases under moisture stress conditions [18]. There could be many direct and indirect adverse effects of low moisture availability to crop plants. One of these adverse effects is fewer uptakes of S and other plant nutrients. Sulfur moves from non-rhizospheric soil to plant roots mainly through mass flow [19] which is adversely affected by low moisture conditions in soil. Thus, insufficient availability of S to a high S requiring crop such as the *Brassica napus*, as evident from S concentrations and S uptake data of the second year's experiment (Table-3), could be one of the main reasons for decline in crop growth and yield.

A significant increase in oil content even at 10 kg S ha^{-1} indicates that the effect of S fertilization is stronger on oil formation as compared to the vegetative growth in the *Brassica napus* [4, 5]. Increasing rate of S fertilization increases its availability and utilization by crop plants, which improves the overall photosynthetic activity in plants resulting in production of more biomass and crop yields [5].

On the whole, S applied at 30 kg ha^{-1} proved to be the optimum level of S fertilization under the agro-climatic conditions of this study. These results are in line with the earlier findings, according to which 30 kg S ha^{-1} was the most suitable and appropriate dose for increasing *Brassica napus* yield in S deficient soil [20]. Many scientists have recorded maximum S uptake between 20 and 30 kg ha^{-1} by the oilseed crops [21, 22]. In the present study however, the maximum S uptake of 17.6 kg ha^{-1} (mean 14.4 kg ha^{-1} at both the Rawal and Therpal soils) was recorded at Rawal soil with the application of ammonium sulfate at 40 kg S ha^{-1}. Hence, the uptake of S by *Brassica napus* plants in this study has been comparatively low. The reasons could be less availability of moisture through insufficient winter rains and less total biomass production at the experimental sites in this study. Inadequate moisture availability in soil due to unpredictable, erratic and insufficient rains during most part of the year was regarded to be the main reason of low fertilizer use efficiency and low productivity of the rain-fed Pothwar [15].

Rate of Sulfur Application

Table-3: Main effects of S application rate (kg ha^{-1}), sources of S, locations and year on salique plant^{-1}, biomass yield, seed yield, oil concentrations, S concentrations and S uptake of the *Brassica napus* crop in two year field experiment.

Treatments	Salique plant^{-1}	Biomas yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Oil content (%)	S contents (mg g^{-1} dry matter)	S uptake (kg ha^{-1})
Rates of S (kg ha^{-1})						
0	286 d	8904 d	1581 c	40.7 d	0.90 d	8.0 d
10	293 cd	8943 c	1597 bc	42.5 c	1.02 c	9.2 c
20	303 bc	8993 b	1620 b	43.4 b	1.22 b	11.0 b
30	314 ab	9054 a	1655 a	44.6 a	1.54 a	13.9 a
40	318 a	9058 a	1656 a	44.5 a	1.56 a	14.1 a
LSD value	11.6**	32.9**	24.2**	0.51**	0.02**	0.99**
Sources of S						
SSP	300	8976	1612^{NS}	43.0^{NS}	1.22 ab	11.0 b
AS	313	9033	1651	43.8	1.41 a	12.9 a
G	295	8962	1603	42.4	1.16 b	10.4 b
LSD value	NS	NS	NS	NS	0.21*	1.83*
Rates × Sources Interaction						
LSD value	NS	82.0*	NS	0.88**	0.03*	2.86**
Locations						
Rawal	314 a*	9201 a**	1740 a**	44.2^{NS}	1.32^{NS}	12.2 a*
Therpal	292 b	8780 b	1503 b	41.9	1.20	10.6 b
Years						
2004-2005	322^{NS}	9134 a**	1719 a**	43.4^{NS}	1.34^{NS}	12.3 a*
2005-2006	283	8847 b	1525 b	42.8	1.17	10.4 b

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$). NS stands for non significant difference, SSP stands for single super phosphate, AS for ammonium sulfate and G for gypsum. * and ** denote significance at $P < 0.05$ and $P < 0.01$ levels, respectively.

Sources of S

In the present study, all the three S sources proved equally effective in promoting growth and yield of *Brassica napus* crop during both years of the study, and therefore verified the previous findings [23, 24]. Higher S uptake in AS treated plots might be due to relatively higher solubility of AS as compared to SSP and G [25, 26]. Ammonium sulfate has been found effective in promptly alleviating crop S deficiency when applied in time [27]. On the other hand, S availability from G to plants is primarily controlled by its sparingly soluble nature [26]. A significant response of S applied as AS has been reported in oilseeds by many researchers [28, 29]. Ammonium sulfate has been found to be superior to G and pyrites in its direct, residual and cumulative effects on the oilseed crops [30]. Contrary to these findings, there was not any significant difference between AS and gypsum as the S source for oilseed crops and the yield obtained by S applied through both of these sources was statistically similar to each other [23]. However in some studies under high rainfall conditions, G was reported to be superior to both SSP and AS due to less leaching losses from G [31]. In another study, AS was found to be inferior to G and SSP due to low retention capacity and high leaching of sulfate in irrigated condition in course textured soils poor in organic carbon [32]. Hence, it can be concluded that effectiveness of different S sources varies with soil texture, climatic condition and test crop used in the study.

Soil S fractions

The order of incorporation of applied S into different soil fractions was: organic S > soil solution S > adsorbed S. This indicated importance of soil organic matter as the major sink for the S applied to soil [33, 34]. These results are in conformity with the results of trial carried out in New Zealand where application of SSP at the rate of 376 kg ha^{-1} for 50 years resulted in increase in both organic (363 to 531 kg ha^{-1}) and inorganic S (1.4 to 7.2 kg ha^{-1}) [35]. Contrary to these findings, annual application of inorganic S for 150 years had very little effect on different soil S fractions of arable soil from the Broadbalk experiments [8, 9]. The difference may be explained in several ways. Major reason for difference in results from Broadbalk experiments may be due to difference in soil organic carbon. Our experimental sites were much poorer in organic carbon and in a soil where organic carbon is accumulating, there is much more possibility of increase in organic S [8]. Almost similar results were recorded in India where the status of total, organic and inorganic S improved in plots that received graded rates of S [36]. The contribution of AS to $CaCl_2$-extractable S and the adsorbed S fractions was lowest among the S sources which could be related to more leaching losses of S from this source [25]. On the other hand, SSP and G are relatively less subject to leaching losses due to slow release of sulfate from these sources, and therefore were able to contribute more to soluble and adsorbed S fractions in the soil [24, 26].

Economic Analysis

Although there was a non-significant difference between the *Brassica napus* yields obtained though SSP and G, but former was more economical S source than latter. Higher net return with AS may be related to its higher solubility and better S availability to *Brassica napus* plants from this source under moisture stress conditions. The presence of readily soluble S at higher concentrations might be more prone to losses. On the other hand, gypsum being the cheapest source of S was also capable of providing economical returns at 30 S kg ha^{-1} application rate along with its known favorable effects on soil physical properties. Nevertheless, the cost of G is very low (Rs. 2.0 kg^{-1}) in Pakistan, but the main reason for limited use of G as a fertilizer is its relatively low nutrient analysis [37]. Gypsum adds only a single nutrient to soil as compared to AS or SSP which add N and P, respectively in addition to S. On the whole, the economics of S fertilizer use in this study clearly suggested that money invested on S fertilizers in *Brassica napus* can bring more return to the farmers of areas under rain-fed dry farming.

Experimental

A two years field experiment was carried out on two different S deficient soils of Pothwar, Pakistan [Rawal (*Sandy loam*, *Ustochrepts*, *Inceptisols*) located at the Research Farm of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan; and Therpal (*Sandy loam*, *Haplustalf*, *Alfisols*) located at Barani Agricultural Research Institute Chakwal, Pakistan]. The physical and chemical characteristics of soils at the experimental sites are given in Table-3.

The experiment was laid out in Randomized Complete Block Design with split plot arrangement. The treatments included three S sources [SSP containing 12% S, AS containing 24% S, and G containing 15% S] each applied at five different rates (0, 10, 20, 30 and 40 kg S ha^{-1}). Sources of S were kept in main plots, while the application rates of S were accommodated in subplots. A uniform basal dose of 90 kg N ha^{-1} as urea and 60 kg P ha^{-1} as triple super phosphate (TSP) were applied to all plots. For the plots receiving S as AS or as SSP, the dose of urea or TSP was adjusted accordingly. Thus, all the treatments received the same amounts of N and P, as the differences in N and P content between the three S-sources was equalized with urea and TSP. *Brassica napus* cultivar *Dunkeld* was sown at both the locations in winter 2004-05, and after one fallow season in winter 2005-06 on the same soils. A composite soil sample was taken from each site before start of experiment and analyzed for physical and chemical properties. Soil texture was determined by hydrometer using sodium hexametaphosphate as dispersing agent and electrical conductivity by HANNA HI-8033 conductivity meter [38]. Soil pH was determined from 1:1 soil water suspension using HANNA 212 pH meter [38]. Soil was analyzed for $CaCO_3$ by titaration against NaOH using phenolphthalein as indicator [39]. Total organic carbon was determined by titration against FeSO4 using diphenyl amine as an indicator [38]. Total nitrogen from soil samples was analyzed by kjeldahl digestion appararus [38].

Plant growth parameters such as plant height and saliquae $plant^{-1}$ were measured just before the crop harvest. Other crop data on biomass and seed yield were recorded after the crop harvest. Plant and seed samples were prepared and analyzed for total sulfur [40] and oil concentration [21].

Total S in Plant Tissue

Ten ml of filtrate prepared by dry ashing was taken in a 150 ml Erlenmeyer flask. One ml of 6 M HCl solution and 5 ml 70 % sorbitol solution were added. Finally about 1 g barium chloride crystals were added in order to develop the turbidity. Absorbance was recorded by using spectrophotometer at 470 nm [40].

Oil Concentration Seed

Seed analysis for oil concentration was performed at Oil Quality Laboratory, National Agriculture Research Centre, Islamabad with Nuclear Magnetic Resonance Spectrometer [21].

The soil samples collected from 30cm depth from each treatment after the harvest of crop plants were air-dried, finely ground to pass a 100-mesh sieve and analysed for different S fractions.

Total S in Soil

Total S was determined by wet oxidation [41]. Wet oxidation involves oxidation of soil S with NaOBr for conversion of soil S to sulphate (oxidized S). The sulphate was then determined by turbidimetric procedure using barium choride [40].

The 0.01 M $CaCl_2$-extractable SO_4^{-2}-S and 0.016 M KH_2PO_4-extractable SO_4^{-2}-S were determined by turbiditimetric method using *Cecil-2000* spectrophotometer (Cecil instruments Cambridge England) as described below.

$CaCl_2$ Extractable Soil Sulfur

A five gram soil sample was taken in a 150 ml Erlenmeyer flask. Twenty five ml of 0.15 % $CaCl_2.2H_2O$ solution was added into flask. Flask was shaken for about 30 minutes and then contents were filtered. Ten ml of filtrate was taken in 50-ml volumetric flask along with 1 ml of 6 M HCl solution and 5 ml 70 % sorbitol solution. Finally about 1 g barium chloride crystals were added in order to develop the turbidity. Absorbance was recorded by using *Cecil -2000* spectrophotometer at 470 nm (Cecil instruments Cambridge England) [40].

KH_2PO_4 Extractable Soil Sulfur

A solution of KH_2PO_4 containing 500 mg l^{-1} of P was used for extraction of soluble and adsorbed sulphate in soils. This solution extracts adsorbed sulphate effectively from most soils. Soil sample was shaken with KH_2PO_4 solution, filtered and S was determined turbidimetrically as described in previous section for $CaCl_2$ extractable soil sulfur [40].

The adsorbed S was estimated as the difference between KH_2PO_4-extractable SO_4^{-2}-S and the $CaCl_2$-extractable SO_4^{-2}-S [17] while the soil organic S was estimated as the difference between total S and $CaCl_2$-extractable SO_4^{-2}-S [42]. Moisture concentrations in the air-dried soil samples were estimated in order to express the results on oven dry soil basis.

The economic analysis of crop response to different rates and sources of S were calculated through VCR and MRR. The statistical analyses of the experimental data were carried out by applying analysis of variance and least significant difference test techniques using softare MSTATC (MSTATC, East Lansing, Mich.) [43].

Conclusion

Yield of oilseed rape can be increased by the application of S fertilizers in S deficient soils by at least 10 percent. All the three S fertilizers sources increased the *Brassica napus* yield in order of AS>SSP>gypsum. The successive application of S fertilizers resulted in build up of Soil S pool (both organic and inorganic) as total S in soil increased from 80 ug g^{-1} in control to 91 ug g^{-1} with application of 40 kg S ha^{-1}.despite growing high S-requiring crop in soils prone to high leaching losses Thus, it is recommended that regular S applications may be practiced for sustainable crop production of high S requiring crops in this as well as other similar areas.

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References

1. M. Hatam and G. Q. Abbasi, Oil Seed Crops, in S. Nazir, E. Bashir, and R. Bantle (Eds) Crop Production. National Book Foundation, Islamabad, Pakistan. pp. 374 (1994).
2. Government of Pakistan, Economic Survey 2010-11. Finance Division, Islamabad Pakistan. p. 20. (2011).
3. H. Marschner, Mineral nutrition of higher plants, Academic Press New York, 889 p. (1995).
4. S. P. McGrath and F. J. Zhao, *Journal of Agricultural Science*, **126**, 53 (1996).
5. J. L. Havlin, J. D. Beaton, S. L. Tisdale and W. L. Nelson, Soil fertility and fertilizers. 7th ed. Pearson Education Singapore, 422 p (2004).
6. H. J. Jr. Mascagni, S. A. Harrisom and G. B. Padgett, *Communication in Soil Science and Plant Analysis*, **39**, 2133 (2008).
7. H. W. Scherer, *Journal of Plant Nutrition and Soil Science*, **172**, 326 (2009).
8. J. S. Knights, F. J. Zhao, B. Spiro and S. P. McGrath, *Journal of Environmental Quality*, **29**, 1867 (2000).
9. J. S. Knights, F. J. Zhao, S. P. McGrath and N. Magan, *Soil Biology and Biochemistry*, **33**, 1797 (2001).
10. Z. Yang, B. R. Singh, S. Hansen, Z. Hu and H. Riley, *Soil Science Society of America Journal*, **71**, 163 (2007).
11. R. J. Haynes and P. H. Williams, *Soil Biology and Biochemistry*, **24**, 209 (1992).
12. N. G. Riley, F. J. Zhao and S. P. McGrath, *Soil Use and Management*, **18**, 120 (2002).
13. D. M. Hedge and I. Y. L. N. Murthy, *Indian Journal of Fertilizers*, **1**, 93 (2005).
14. F. Hassan, A. Manaf, G. Qadir and S. M. A. Basra, *International Journal of Agriculture and Biology*, **9**, 504 (2007).
15. K. S. Khan and R. G. Joergensen, *Journal of Plant Nutrition and Soil Science*, **169**, 494 (2006).
16. F. J. Zhao, S. P. McGrath, M. M. A. Blake-Kalff, A. Link and M. Tucker, *Crop Responses to Sulphur Fertilisation in Europe*, in Proc.of International Fertilizer Society No. 504, International Fertilizer Society, York, UK. 27 p. (2002).
17. R. Khalid, K. S. Khan, G. Shabbir, M. Yousaf and S. Y. Naz, *Pakistan Journal of Scientific and Industrial Research*, **52**, 84 (2009).
18. R. A. Richards and N. Thurling, *Australian Journal of Agricultural Research*, **29**, 479 (1978).
19. Z. Y. Hu, F. J. Zhao and S. P. McGrath, *Plant and Soil*, **268**, 103 (2005).
20. L. Shutian, B. Lin and W. Zhao, Crop Response to Sulfur Fertilizers and Soil Sulfur Status in Some Provinces of China, in: L. J. D. Kok, and E.Schnug (Eds.) Sulfur Nutrition of Plants. Proceedings of Ist Sino-German Workshop on Aspects of S Nutrition of Plants in May in Shenyang, China. p. 81. (2005).
21. J. Warnesly, *Lipid Technology*, **10**, 6 (1988).
22. S. S. Malhi and K. S. Gill, *Canadian Journal of Plant Science*, **82**, 665 (2002).
23. N. Ahmad, M. T. Saleem, M. Rashid and A. Jalil, Sulfur status and crop response in Pakistan soils. Planning and Development Division, Islamabad-Pakistan, p. 3 (1994).
24. P. Ryant and J. Skladanka, *Acta Agriculture Scandinavica*, *Section B*, *Soil and Plant Science*, **59**, 208 (2009).
25. H. W. Scherer, *European Journal of Agronomy*, **14**, 81 (2001).
26. G. Wen, J. J. Schoenau, , S. P. Mooleki, , S. Inanaga, T. Yamamoto, , K. Hamamura, , M. Inoue and P. An, *Journal of Plant Nutrition and Soil Science*, **166**, 54 (2003).
27. P. J. A. Withers, A. R. J. Tytherleign and F. M. Odonnell, *Journal of Agricultural Science*, **125**, 317 (1995).
28. Rahmatullah, G. Nabi, M. Salim and M. S. Zia, *Pakistan Journal of Biological Sciences*, **2**, 462 (1999).
29. A. Subhani, G. Shabir, M. Fazil, A. Mahmood, R. Khalid and N. M. Cheema, *Pakistan Journal of Soil Science*, **22**, 50 (2003).
30. G. Nabi, Rahmatullah and M. Salim, *Pakistan Journal of Soil Science*, **26**, 460 (1990).
31. B. Parsad, *Journal of Plant Nutrition*, **26**, 997 (2003).

32. A. S. Raju, *Sulfur Status and Responses of Crops to Applied Sulfur in Red Soils (Altisols) of Andhra Pradesh*, *Importance of Sulfur in Balanced Fertilizer Use*, in Proc. of TSI-FAI Workshop on Importance of Sulfur in Balanced Fertilizer Use in May 1994 in New Delhi, India, p. 17 (1994).
33. S. Haneklaus, E. Bloem and E. Schnug, Sulfur, in R. Lal (Ed.) Encyclopaedia of Soil Science, 2nd edition, CRC Press, 2060 p. (2005).
34. Z. Hu, Z. Yang, C. Xu, S.Haneklaus, Z. Cao and E. Schnug, *Journal of Plant Nutrition and Soil Science*, **165**, 249 (2002).
35. K. M. Goh and J. Pamidi, *Plant and Soil*, **264**, 299 (2004).
36. K. S. Reddy, M. Singh, A. K. Tripathi, A. Swarup and A. K. Dwivedi, *Australian Journal of Soil Research*, **39**, 737 (2001).
37. D. L. Messick, M. X. Fan and C. D. Brey, *Global Sulfur Requirements and Sulfur Fertilizers*, in: L. J. D. Kok and E. Schnug (Eds.) Sulfur Nutrition of Plants, Proc. of the Ist Sino-German workshop on aspects of S nutrition of plants in May 2005 in Shenyang, China, p. 97 (2005).
38. A. L. Page, R. H. Miller and D. R. Keeney, Methods of Soil Analysis, Part 2. 2nd edition American Society of Agronomy, Monograph No. 9, Madison, Wisconsin, USA. pp. 506-509. (1982).
39. J. Ryan, G. Estefan and A. Rashid. Soil and Plant Analysis Laboratory Manual. 2nd Edition. International Center for Agricultural Research in Dry Areas (ICARDA) Aleppo, Syria. pp.118-127. (2001).
40. B. C. Verma, K. Swaminathan and K. C. Sud, *Talanta*, **24**, 49 (1977).
41. M. A. Tabatabai, Sulfur, in A. L Page, J. R. Freney and R. H. Miller (Eds.) Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. ASA and SSSA, Madison, WI USA, p.501 (1982).
42. M. L. Nguyen and K. M. Goh, *Biology and Fertility of Soils*, **14**, 181 (1992).
43. R. R. Sokal and F. J. Rohlf, Biometry: The Principles and Practices of Statistics in Biological Research. 4th edition, W. H. Freeman and Co., New York, 937 p. (2012).