

Research Article

Sulphur fertilization on biochemical constituents of cabbage (*Brassica oleracea var. capitata*. L) in non-calcareous soil of Coimbatore district, Tamil Nadu

S. Roshini*

Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India

D. Jegadeeswari

Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India

T. Chitdeshwari

Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India

A. Sankari

Department of Vegetable Science, HC& RI, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India

*Corresponding author. Email: sroshinirose@gmail.com

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Abstract

Sulphur is the fourth most important plant nutrient after nitrogen, phosphorus, and potassium, and it is becoming increasingly crucial in high-quality crop production (Bhojar., 2019). Since limited work has been carried out regarding different sulphur sources on cabbage production in the Coimbatore district, the present study was undertaken to investigate the sulphur sources and levels on various biochemical constituents of cabbage. Hence a field experiment was conducted in the farmer's field at Viraliyur village, Thondamuthur block of Coimbatore district, Tamil Nadu to assess the effect of sulphur fertilization on improving the biochemical constituents of cabbage hybrid Saint. There were four different S sources (Elemental sulphur, Potassium sulphate, Gypsum, Single super phosphate) applied at five levels (0, 20, 40, 60 and 80 kg ha⁻¹) and replicated thrice in a factorial randomized block design. The crop was fertilized with a Soil Test Crop Response-prescribed dose of NPK (200:125:25 kg ha⁻¹). The crop was harvested on 90th day and cabbage heads were analysed for various biochemical constituents like ascorbic acid, total soluble solids (TSS), titratable acidity (TA), chlorophyll content, glucosinolates (GLs), total phenol content (TPC), sulphur containing amino acid (methionine) and antioxidant enzyme activity (peroxidase). The influence of S fertilizers on biochemical constituents increased significantly with increasing levels of sulphur fertilization up to 80 kg S ha⁻¹ excluding ascorbic acid content. The pooled data showed that gypsum applied at 80 kg ha⁻¹ registered the maximum GLs (69.0 µmol g⁻¹), TPC (31.9 mM 100g⁻¹), methionine (32.3%), peroxidase activity (0.70 units min⁻¹mg⁻¹), TSS (7.64 °Brix), TA (0.64%), ascorbic acid (61.4 mg 100g⁻¹) and total chlorophyll (1.21 mg g⁻¹) in cabbage head. The lowest content of biochemical constituents viz., GLs (34.1 µmol g⁻¹), TPC (8.10 mM 100g⁻¹), methionine (17.6%) and peroxidase (0.31 units min⁻¹mg⁻¹) were observed in control applied NPK alone. There were positive and significant changes in the biochemical constituents of cabbage due to S application which confirms the improvement in the quality of cabbage head. The study concluded that gypsum was the better sulphur source for improving the quality of cabbage.

Keywords: Biochemical constituents, Cabbage, Levels, Sources, Sulphur

INTRODUCTION

In India, nearly 42% of the cultivated soils are deficient in available sulphur (S) and good yield responses to S

application have been reported by Prasad and Shivay.,2017 Sulphur plays a key role in improving the growth, production, productivity, and quality of crops (Bhat *et al.*,2017). After nitrogen, phosphorus and po-

tassium, the sulphur is considered the fourth important macronutrient (Jamal *et al.*, 2010) which is required for normal growth of plants. Sulphur is regarded as an essential nutrient for protein metabolism, synthesis of oil and formation of amino acids (Krishnamoorthy, 1989) and it is one of the constituents of essential amino acids such as cysteine (26% S), cystine (27% S) and methionine (21% S) and thus in proteins, (Hunashikatti *et al.*, 2000a; Singh *et al.*, 2017; Verma and Nawange 2015). Cabbage is one of the significant and nutritious winter cruciferous leafy vegetables grown widely in India. It contains important vitamins, minerals, and trace amounts of protein, all of which have a better caloric value. White cabbage (*Brassica oleracea var. capitata* L.) is consumed worldwide and is considered a good source of bioactive phytochemicals. Sulphur application is necessary to increase production and productivity while also improving cabbage quality (Bhat *et al.*, 2017). The content of glucosinolates is affected by several environmental factors such as temperature, light, soil type and fertilizer applications (Martinez-Ballesta *et al.*, 2013)

Brassica crop contains high sulphur content relative to other crops, and a lack of sulphur reduces crop productivity and quality. Its uptake and distribution is controlled in response to changes in nutrient demand (Yoshimoto *et al.*, 2003; Buchner *et al.*, 2004). Sulphur performs as primary and secondary component of cysteine and methionine synthesis, Vit B₁ and H, enzymes and coenzymes (Prasad. & Shivay, 2017). Sulphur application lowers the soil pH there by increasing the availability of several essential nutrients and results in the activation of number of enzymes, energy transformation, chlorophyll formation and carbohydrate metabolism (Bairwa *et al.*, 2017). Since, Indian soils are deficient in available sulphur, the requisite quantity for different crops needs to be applied as sulphur fertilization through various sources and levels. Cabbage is one of the preferred vegetables and sulphur loving crop which ought to be supplied with sulphur for its normal growth, yield and quality. Hence, this study was carried out with various levels of sulphur sources to assess the changes in biochemical constituents in the cabbage head to determine the quality improvement.

MATERIALS AND METHODS

Field experiment

To study the impact of various sources and levels of sulphur on various biochemical constituents, a field experiment with cabbage (*Brassica oleracea var. capitata* L.) was conducted in a farmer's field at Viraliyur, Thondamuthur, Coimbatore district, Tamil Nadu, during rabi season in the year of February to May 2020. The initial soil analysed was red sandy loam in texture with deficient S and low organic carbon and having pH 8.1.

Cabbage hybrid Saint was supplemented with four different S sources *viz.*, elemental sulphur, potassium sulphate, gypsum and single super phosphate (SSP) applied at five levels as 0, 20, 40, 60 and 80 kg ha⁻¹ which were replicated thrice in Factorial randomised block design (FRBD). The crop was fertilized with the STCR prescribed dose of NPK (200:125:25 kg ha⁻¹) by following standard cultural practices as outlined by TNAU (HPG, 2020) and the crop was harvested on the 90th day. Cabbage head was collected from all the treatments and analysed for biochemical constituents such as ascorbic acid, total soluble solids, titratable acidity, chlorophyll content, glucosinolates, total phenol content, sulphur-containing amino acid (methionine) and antioxidant enzyme (peroxidase). The details of experimental techniques and methodology used for individual parameters were described below.

Analysis of biochemical (quality) constituents

Ascorbic acid

Ascorbic acid content was estimated by the titrimetric method using 2, 6-dichlorophenol indophenol dye. The Appearance of light pink colour shows the presence of ascorbic acid content. It is expressed as mg 100g⁻¹ of the fresh sample as outlined by Pearson (1976).

Titratable acidity

Titratable acidity was determined by using the methodology described by Instituto Adolfo Lutz (2008) and expressed in percentage.

Total soluble solids (TSS)

Finely chopped cabbage heads were macerated and the extracted juice was filtered and volume was made up to 250 ml. Total soluble solids were assessed using a hand refractometer and represented by °Brix.

Chlorophyll pigments

Total chlorophyll content was estimated using the method proposed by Arnon (1949). Fifty milligrams of fresh leaf material was homogenized with 5ml of acetone centrifuged @ 2000 rpm for 10 minutes and volume was made up to 10ml. The absorbance of the supernatant was measured at 645 and 663 nm using a Spectrophotometer (Systronics, India) and the total chlorophyll content was calculated and expressed as mg/g of fresh weight.

Total phenols

Total phenols content was determined by Bray and Thorp's technique (1954), in which fresh leaf samples along with ethanol was heated, cooled and centrifuged at 5000 rpm. Sodium carbonate and Folin's reagents were added and estimated colorimetrically at 660 nm. Standards were prepared using catechol at different concentrations and total phenols content was estimated

and expressed in mg g^{-1} .

Glucosinolates

Homogenized 0.1 g dry sample in a vial with 80% methanol and centrifuged at 3000 rpm and kept overnight at ambient temperature. Then the supernatant was made up with 80% methanol. To 100 μl of extract, 0.3 ml double distilled water and 3 ml of 2 mM sodium tetrachloropalladate (58.8 mg Sodium tetrachloropalladate + 170 μl concentrated HCl +100 ml double distilled water) were added. After incubation at ambient temperature for 1 hr, absorbance was spectrophotometrically computed at 425 nm and the total glucosinolates was calculated (Mawlong *et al.*, 2017).

Methionine

The Nitroprusside sodium method, the simplest method for the photometric determination of methionine suggested by McCarthy and Sullivan (1941) was followed. One ml of 14.3 M sodium hydroxide solution, 1 ml of 1% glycine solution and 0.3 ml of 10% nitroprusside sodium solution were added to 5 ml of the test solution, placed in a water bath at 35-40 $^{\circ}$ C for 5 to 10 minutes, cooled with a mixture of water and ice at 0 $^{\circ}$ C. Five ml of a mixture of hydrochloric acid and phosphoric acid (8 parts of concentrated hydrochloric acid and 1 part of 85% phosphoric acid by volume) was added with constant stirring. After vigorous stirring, cooled in water and kept at room temperature for 5 to 10 minutes. The absorbance of the samples was measured at 580 nm.

Peroxidase

Leaf samples were homogenised in a phosphate buffer, and 1 ml of the supernatant was obtained, along with 3 ml of 0.05M pyrogallol and 0.5 ml of 30% hydrogen peroxide were added. The change in absorbance was measured at 430 nm for every 30 seconds upto 2 mins and the enzyme activity was calculated and expressed as units $\text{min}^{-1} \text{mg}^{-1}$ of sample (Sadasivam and Manickam, 1992).

Statistical analysis

The results pertaining to the study on biochemical compounds were statistically analysed using AGRESS software version 7.01. The mean values were compared using DMRT and the differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The findings of this study showed that sulphur sources and levels had, in general, a significant influence on cabbage quality, determining biochemical parameters.

Effect of sulphur sources and levels on various biochemical compounds

TSS, TA, Ascorbic acid, Total Chlorophyll content

The effect of sulphur fertilization on TSS ($^{\circ}$ Brix), titratable acidity (%), ascorbic acid ($\text{mg } 100\text{g}^{-1}$) and total chlorophyll content (mg g^{-1}) of the cabbage head showed a statistically significant variation ($P < 0.05$) among all sources of sulphur. The TSS ranged between 5.65 to 7.64 $^{\circ}$ Brix, titratable acidity ranged from 0.23 to 0.64%, ascorbic acid content was recorded between 44.1 to 61.4 $\text{mg } 100\text{g}^{-1}$ and chlorophyll contents were ranged from 0.64 to 1.21 mg g^{-1} , respectively. Gypsum as one of the sources of sulphur recorded the maximum mean of TSS (7.02 $^{\circ}$ Brix), TA (0.49%), ascorbic acid (53.9 $\text{mg } 100\text{g}^{-1}$), and total chlorophyll content (0.94 mg g^{-1}) followed by single super phosphate which recorded TSS (6.47 $^{\circ}$ Brix), TA (0.40%), ascorbic acid (51.8 $\text{mg } 100\text{g}^{-1}$) and total chlorophyll (0.88 mg g^{-1}) (Table 1 and 2; Fig. 1 and Fig. 2).

Further, the study revealed that among the different levels of sulphur irrespective of sources, 80 kg ha^{-1} recorded the highest total soluble solids (6.91 $^{\circ}$ Brix), titratable acidity (0.52%) and chlorophyll content (1.11 mg g^{-1}), but for vitamin C (59.6 $\text{mg } 100\text{g}^{-1}$) which showed maximum content under 60 kg ha^{-1} of sulphur. These observations were in accordance with Kusz-

Table 1. Effect of sulphur sources and levels on ascorbic acid content in cabbage head

Sulphur sources / Levels (kg ha^{-1})	Ascorbic acid ($\text{mg } 100\text{g}^{-1}$)					Mean
	0	20	40	60	80	
Elemental sulphur	44.1 ^{cd}	47.0 ^c	50.3 ^{bc}	59.0 ^{ac}	52.4 ^{bc}	50.6
Potassium sulphate	44.3 ^{bcd}	48.6 ^{bc}	52.9 ^{bc}	58.6 ^{a^{bc}}	52.7 ^{bc}	51.4
Gypsum	45.5 ^{ad}	51.0 ^{ac}	57.5 ^{ab}	61.4 ^a	54.2 ^{ab}	53.9
SSP	44.2 ^{bd}	49.0 ^{bc}	53.0 ^b	59.6 ^{ab}	53.0 ^b	51.8
Mean	44.5	48.9	53.4	59.7	53.1	51.9
SEd	0.47		0.53		1.06	
CD (P=0.05)	0.96		1.07		2.14	

Table 2. Effect of sulphur sources and levels on total soluble solid contents in cabbage head

Sulphur sources / Levels (kg ha ⁻¹)	Total soluble solids (°Brix)					Mean
	0	20	40	60	80	
Elemental sulphur	5.65 ^{de}	6.17 ^d	6.33 ^{cd}	6.38 ^{bd}	6.42 ^{ad}	6.19
Potassium sulphate	5.78 ^{ce}	6.28 ^{cd}	6.40 ^c	6.51 ^{bc}	6.62 ^{ac}	6.32
Gypsum	6.23 ^{ae}	6.68 ^{ad}	7.14 ^{ac}	7.39 ^{ab}	7.64 ^a	7.02
SSP	5.87 ^{be}	6.32 ^{bd}	6.57 ^{bc}	6.67 ^b	6.94 ^{ab}	6.47
Mean	5.88	6.36	6.61	6.74	6.91	6.50
SEd	0.04		0.04		0.09	
CD (P=0.05)	0.08		0.09		0.18	

nierewicz *et al.* (2008) results, who conducted experiments on white cabbages to quantify the phenolic compounds, carotenoids, ascorbic acid, and antioxidant potential under different sources of sulphur. Sulphur forms the components of secondary metabolites, which play an important role in improving the quality of the produces. Higher chemical and biological activation of iron content in leaves might have increased the chlorophyll content (Bhat *et al.*, 2017; Singh *et al.*, 2017).

Glucosinolates (GLs)

The glucosinolate content in the cabbage head was significantly ($P < 0.05$) influenced by S nutrition from different sources and the content varied from 34.1 to 69.0 $\mu\text{mol g}^{-1}$. Increasing levels of sulphur increased the glucosinolate contents in the cabbage head. Among the different levels of sulphur, the highest glucosinolate content of 62.5 $\mu\text{mol/g}$ was recorded in the treatment applied with 80 kg ha⁻¹ of S, followed by 60 kg ha⁻¹ of S and the lowest content of 35.3 $\mu\text{mol g}^{-1}$ was recorded in the treatment without S (Table 3). Among the sources of S, gypsum application (52.1 $\mu\text{mol g}^{-1}$) recorded the maximum glucosinolate content, which was followed by SSP (49.4 $\mu\text{mol g}^{-1}$), potassium sulphate (46.3 $\mu\text{mol g}^{-1}$) and elemental S (43.0 $\mu\text{mol g}^{-1}$). These results are in accordance with earlier research works by Zaki *et al.* (2009), Bimova and Pokluda (2009), who increased the development of Brassica plants (broccoli and cabbage)

by supplementing N and S. Brassica plants exposed to SO₂ and H₂S produced more water-soluble, non-protein thiol compounds in the shoot, which might be the reason for increased glucosinolate concentration (Buchner *et al.*, 2004; Shahbaz *et al.*, 2014). The increased gypsum concentration enhanced the total and indo-lyl glucosinolates in broccoli (Rangkadilok *et al.*, 2004).

Total phenol (TPC)

Total phenols in cabbage heads were significantly ($P < 0.05$) influenced by sulphur fertilization and the content varied from 8.10 to 31.9 mM of fresh weight (100 g⁻¹) (Table 4). The highest TPC in cabbage head was recorded by the sulphur level of 80 kg ha⁻¹ of sulphur applied as gypsum (31.9 mM 100g⁻¹) followed by SSP (28.4 mM 100g⁻¹). The lowest total phenol content of 8.10 mM 100g⁻¹ was registered in control plants with no sulphur applied. Among the different sources of S, gypsum recorded the highest mean of 21.4 mM 100 g⁻¹ for TPC followed by SSP (19.3 mM 100 g⁻¹), potassium sulphate (18.1 mM 100g⁻¹) and elemental sulphur (16.7 mM 100g⁻¹). The favourable effect of S, might have enhanced the growth of cabbage seedlings and also contributed to an increase in phenolic content in cabbage head. This is in corroboration with the results of Joseph and Raj (2010) who found flavonoids and polyphenolic chemicals contained in the cabbage plant are responsible for the plant's antioxidant properties. The antiradical

Table 3. Effect of sulphur sources and levels on glucosinolates content in cabbage head

Sulphur sources / Levels (kg ha ⁻¹)	Glucosinolates ($\mu\text{mol g}^{-1}$)					Mean
	0	20	40	60	80	
Elemental sulphur	34.1 ^{de}	37.7 ^d	42.4 ^{cd}	48.2 ^{bd}	52.6 ^{ad}	43.0
Potassium sulphate	35.0 ^{ce}	38.8 ^{cd}	44.0 ^c	52.1 ^{bc}	61.6 ^{ac}	46.3
Gypsum	36.7 ^{ae}	44.4 ^{ad}	50.3 ^{ac}	60.2 ^{ab}	69.0 ^a	52.1
SSP	35.4 ^{be}	40.4 ^{bd}	46.2 ^{bc}	58.5 ^b	66.7 ^{ab}	49.4
Mean	35.3	40.3	45.7	54.8	62.5	47.7
SEd	0.01		0.01		0.01	
CD (P=0.05)	0.01		0.01		0.02	

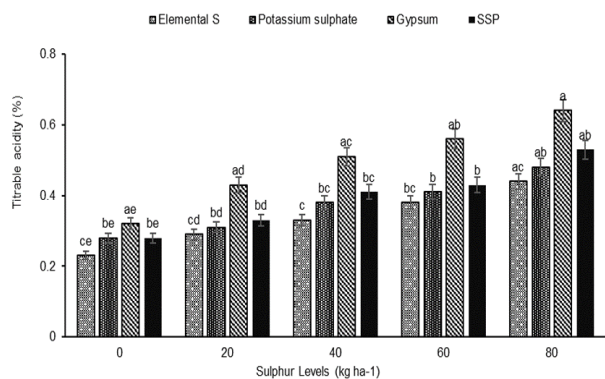


Fig. 1. Effect of different sulphur levels and sources on the titratable acidity (%)

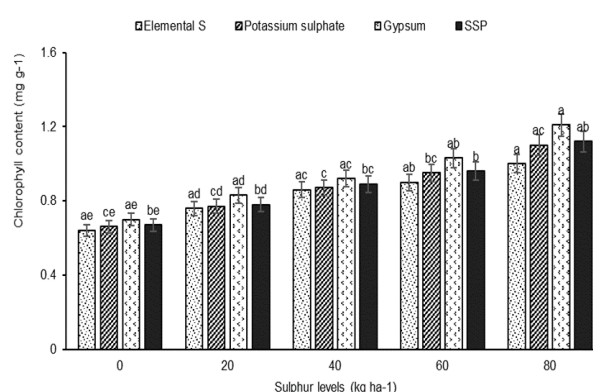


Fig. 2. Effect of different sulphur levels and sources on the chlorophyll content (mg g^{-1})

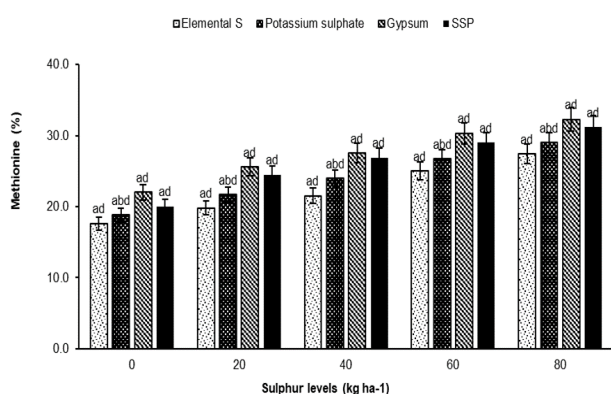


Fig. 3. Effect of different sulphur levels and sources on methionine content (%)

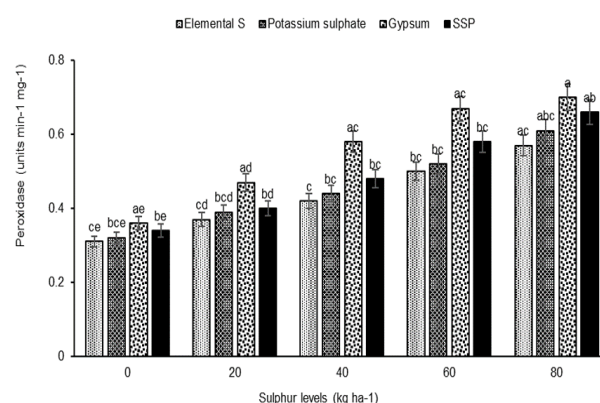


Fig. 4. Effect of different sulphur levels and sources on peroxidase activity ($\text{units min}^{-1} \text{mg}^{-1}$)

scavenging activity of phenolics is associated with the substitution of hydroxyl groups in the aromatic rings of phenolics and the sulphur contributes to their hydrogen-denoting ability (Yen *et al.*, 2005; Evans *et al.*, 2006).

Amino acids

Similar to glucosinolates, total phenols and peroxidase, the S containing amino acid, methionine were also influenced by S fertilization and showed a similar trend in increasing the content due to different sources and levels of S. Increasing levels of S, increased the

methionine content and the values varied from 17.6 to 32.3% in cabbage head (Fig.3). Among the different levels applied, sulphur application @80 kg ha⁻¹ was found superior compared to other levels. The highest methionine content of 32.3% was recorded at 80 kg ha⁻¹ of gypsum as a source of S and the lowest methionine was observed in control plants (17.6%). Among different sources, gypsum recorded the highest content of methionine (27.9%), followed by SSP (26.3%), potassium sulphate (24.0%) and elemental sulphur (22.3%). The lowest value (17.6%) was recorded in the control

Table 4. Effect of sulphur sources and levels on total phenol content in cabbage head

Sulphur sources / Levels (kg ha^{-1})	Total phenol ($\text{mM } 100\text{g}^{-1}$)					Mean
	0	20	40	60	80	
Elemental sulphur	8.10 ^{de}	11.9 ^d	17.0 ^{cd}	21.5 ^{bd}	25.0 ^{ad}	16.7
Potassium sulphate	8.32 ^{ce}	13.1 ^{cd}	19.3 ^c	23.3 ^{bc}	26.5 ^{ac}	18.1
Gypsum	9.90 ^{ae}	17.1 ^{ad}	22.1 ^{ac}	26.1 ^{ab}	31.9 ^a	21.4
SSP	8.54 ^{be}	14.4 ^{bd}	20.4 ^{bc}	24.6 ^b	28.4 ^{ab}	19.3
Mean	8.72	14.1	19.7	23.9	28.0	18.9
SEd	0.03		0.03		0.07	
CD (P=0.05)	0.06		0.07		0.14	

plot with no sulphur. Nikiforova *et al.* (2003) documented that brassica plants assimilate inorganic sulphate into cysteine, which is then converted to methionine. This reduction process is regulated by nitrogen. Therefore increasing sulphur supply increased the methionine content. The results of this study was in accordance with the findings of Niranjana and Devi (1990), in chilli, Hunashikatti *et al.* (2000b) in cabbage by using sulphur fertilization.

Antioxidant enzymes

Peroxidase activity in cabbage head was significantly ($P < 0.05$) influenced by S additions and it ranged from 0.31 to 0.70 units $\text{min}^{-1} \text{mg}^{-1}$ (Fig.4). The sulphur application at increasing levels increased the peroxidase activity. The highest activity was observed in cabbage heads receiving 80 kg ha^{-1} of S through gypsum application (0.70 units $\text{min}^{-1} \text{mg}^{-1}$) followed by 80 kg ha^{-1} of S as SSP (0.66 units $\text{min}^{-1} \text{mg}^{-1}$). The lowest activity was recorded in control treatment (0.31 units $\text{min}^{-1} \text{mg}^{-1}$). Out of different sulphur sources tested, gypsum recorded the highest peroxidase activity of 0.56 units $\text{min}^{-1} \text{mg}^{-1}$ followed by SSP (0.49 units $\text{min}^{-1} \text{mg}^{-1}$), potassium sulphate (0.46 units $\text{min}^{-1} \text{mg}^{-1}$) and elemental sulphur (0.43 units $\text{min}^{-1} \text{mg}^{-1}$). Higher activity of peroxidase and lignification increased the tolerance to pathogens. Increased peroxidase activity obstructs lipid peroxidation, breakdown of chlorophyll, ethylene synthesis, and senescence, thereby increasing quality and shelf life (Singh *et al.*, 2010).

Conclusion

The present study concluded that sulphur played an important role in plant metabolism and the nutritional value of cabbage. Among all the S sources used, gypsum was found to be the best sulphur source for improving the biochemical constituents responsible for the quality of cabbage. Sulphur supplementation through gypsum @ 80 kg ha^{-1} increased the biochemical constituents *viz.*, total soluble solids, titratable acidity, total chlorophyll, glucosinolates, total phenols, methionine and peroxidase activity of cabbage head. This study revealed that S fertilization helped in improving essential amino acids, soluble solids, antioxidant activity and chlorophyll pigments in cabbage which could be beneficial in improving the nutritional quality and shelf life of cabbage.

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Conflict of interest

The authors declare that they have no conflict of interest.

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