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Exogenously Applied Sulphur Improves Growth, Photosynthetic Efficiency, Enzymatic Activities, Mineral Nutrient Contents, Yield and Quality of *Brassica juncea* L.

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Abstract: Background: Due to increasing domestic and industrial demand, edible oil production is not keeping up with demand. To fill this gap, the productivity of oilseeds can be increased by applying adequate nutrients, particularly sulphur (S), at the crucial growth stage. **Purpose:** The present study aims to explore the best concentration of S for its foliar application on various cultivars of mustard. **Methods**: A factorial randomized pot experiment was conducted to investigate the role of leaf-applied S on growth, physiobiochemistry, yield and quality traits of three cultivars of *Brassica juncea* L. (mustard). Five levels of S viz. 0 (water), 15, 30, 45 and 60 ppm S constituted one variant, and the three cultivars (Chutki, Nath Sona and Rohini) were the other variants. The various levels of S were sprayed at 50 and 70 days after sowing (DAS). The growth and physio-biochemical characteristics were studied at 90 DAS, and yield and quality attributes at 120 DAS (harvest). **Results**: The data indicated that increasing S levels up to 45 ppm S improved all parameters of mustard and thereafter (at the level above 45 ppm S) decreased. Cultivar Nath Sona, followed by Rohini and Chutki, performed best. Among the foliar spray treatment of different levels of S, the application of 45 ppm S increased plant dry weight by 40.21, 35.65 and 30.96%, photosynthetic rate by 28.27, 27.44 and 36.29%, pods of a plant by 15.23, 12.12 and 10.80%, seed yield of a plant by 7.54, 3.89 and 4.91%, oil content by 48.70, 46.31 and 43.15% and oil yield of a plant by 24.56, 23.93 and 22.35% in cultivar Nath Sona, Rohini and Chutki, respectively, compared with their respective water-treated plants. Moreover, the oil was examined by GC-MS technique for its various components. The analysis revealed that there were 36 compounds in the oil of the non-treated plants and 44 compounds in the oil of plants treated with 45 ppm S. The extra compounds resulted from the application of 45 ppm S. **Conclusion:** It may be concluded that two sprays of 45 ppm S proved effective in improving the growth, physio-biochemical characteristics, yield and quality of cultivars of mustard, particularly Nath Sona.

Keywords: growth; mustard; physio-biochemistry; sulphur; yield and quality

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1. Introduction

Sulphur (S) is a crucial mineral nutrient required by plants for growth, development and productivity [1].

Generally, the deficiency of nutrients, especially S, in agricultural soil causes serious defects to plant growth and development, such as stunted growth, poor branching, premature leaf fall, reduced plant biomass, lower photosynthetic pigment synthesis, inhibition of protein synthesis and enzyme activities and decreased the productivity of crops [1]. However, due to the regular cultivation of crops, nutrient depletion occurred in the soil. For example, the concentration of S decreases due to the use of S-free fertilizers and the

reduction in the use of traditional organic manure in cultivated land [1,2]. Consequently, in the last few decades, the requirement of S in agriculture has been widely concerned due to its deficiency in agricultural soil and decreased crop productivity.

Various studies reviewed by [1] suggested that the exogenous application (soil/foliar) of different concentrations/doses of S improves many features of the growth and physiology of crop plants. For instance, applying 200 mg S kg^{-1} soil enhanced plant growth, chlorophyll content, net photosynthesis, stomatal conductance and enzyme activities of wheat [3]. The supplementation of 100 and 200 mg S kg^{-1} soil improved plant biomass, chlorophyll content, antioxidant enzyme activities and osmolytes accumulation in mustard [4]. The supply of 40 and 45 kg S ha^{-1} increased the seed yield and oil content of sesame [5]. Moreover, for better growth, development and yield of important crops, it is necessary to provide a sufficient foliar supply of S to growing crop plants, especially oilseed crops [6,7]. However, there are still considerable limitations in our understanding of the effects of S fertilizer on crop growth and nutrient quality under spray conditions.

Brassica juncea L. (mustard) belongs to the Cruciferae family and is an important oilseed and vegetable crop [8,9]. It is placed third among the oilseed crops of the world after soybean and palm [10,11]. It is an important source of edible oil and comprises crucial fatty acids vital to human health [12]. In addition, the proteins accumulated in mustard seeds contain high levels of S-amino acids, which are essential in food used for feeding livestock. Thus, these S-rich seed proteins could be used in human food products [13,14]. However, due to increasing domestic and industrial demand, edible oil production is not keeping up with demand. Therefore, we carried out a pot experiment to explore the best dose of S for its foliar application on various cultivars of mustard.

2. Materials and Methods

2.1. Plant Material

The seeds of various cultivars of mustard were procured from the seed market of Aligarh. A preliminary experiment was performed to assess seed viability, germination speed percentage and seedling growth of mustard cultivars. Based on these parameters, the best three high-yielding cultivars, namely Chutki, Nath Sona and Rohini, were selected for further experimental purposes. The uniform size and healthy seeds were preferred. Before sowing, seeds were sterilized with mercuric chloride ($HgCl_2$) solution (0.01%) and repeatedly cleaned with double distilled water (DDW) to remove $HgCl_2$ from the seeds.

2.2. Design of Experiment and Preparation of Treatment

This experiment was performed during the winter season under natural environmental conditions in a net house of the Department of Botany, Aligarh Muslim University, Aligarh, India (27°52′ N latitude, 78°51′ E longitude, 187.45 m altitude). The experiment was performed as a randomized factorial design in 25 × 25 cm earthen pots filled with farmyard manure and sandy loam soil in a ratio of 1:4. Five foliar treatments of S, constituted one variant and three cultivars of mustard, namely Chutki, Nath Sona and Rohini, the other. A recommended dose of nitrogen (N), phosphorus (P) and potassium (K) was applied to the soil at 80 kg N + 40 kg P_2O_5 + 40 kg K_2O /ha, i.e., 36 mg N + 17.9 mg P_2O_5 + 17.9 mg K_2O/kg soil [15]. The dose of nutrients was given before seed sowing and also supplemented to seedlings after 30 days after sowing (DAS). Plants were sprayed with S at 0 (water), 15, 30, 45 and 60 ppm S twice using a hand sprayer, with the first spray being given at 50 DAS and the second spray at 70 DAS. The application of urea for N, superphosphate for P and muriate of potash for K [10]. The source of leaf-applied S was sodium sulphate [16]. The source of double distilled water comprises zero levels of S. There were four replicates of each treatment. The sampling was done at 90 DAS to study growth, physio-biochemical and microscopic parameters. The yield and quality characteristics at harvest (120 DAS).

2.3. Growth Characteristics

Shoot length was measured in cm, branches and leaves were counted in number, the area of a leaf and a plant was measured in $cm^{2,}$ and fresh and dry weights were expressed in g.

2.4. Physio-Biochemical Parameters

2.4.1. Chlorophyll Content

The chlorophyll content of intact leaves was estimated with the help of SPAD-502 (KMS, Inc., Osaka, Japan).

2.4.2. Gas Exchange Parameters

Under natural environmental conditions, the gas exchange parameters such as net -photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E) of plant leaf were assessed with the help of an Infrared Gas Analyzer (LI-COR-6400, Lincoln, NE, USA).

2.4.3. Relative Water Content (RWC)

The fresh leaf discs were quickly weighed and saturated in deionized water in Petri dishes for 24 h in the dark. The water molecules that adhere to the discs were blotted, and the turgor mass was calculated. The leaf discs were dehydrated for 48 h at 80 °C, and dry mass was determined [17]. The RWC of the leaf was estimated by the following formula:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgor mass} - \text{Dry weight}} \times 100$$

2.4.4. Carbonic Anhydrase Activity (CA)

The leaf CA activity was examined using the procedure of [18]. Leaf samples (200 mg) were chopped into small pieces and placed in Petri dishes containing 0.2 M solutions of cysteine hydrochloride (10 mL). After twenty minutes, these leaf pieces were taken, blotted, and placed in a test tube, then phosphate buffer (pH 6.8), sodium bicarbonate solution and bromothymol blue were added. The test tube was agitated and then left for twenty minutes at 4 °C. The methyl red was used as an indicator, and the reaction mixture was titrated against 0.05N HCl. The CA activity was demonstrated as mol CO_2 kg^{-1} (leaf FM) s^{-1}.

2.4.5. Nitrate Reductase Activity (NR)

The leaf NR activity was estimated following the method described by [19]. The fresh leaf sample (200 mg) was chopped into pieces and put into test tubes, and then phosphate buffer (pH 7.5), isopropanol solutions and potassium nitrate were added. The reaction mixture was left for 2 h at 30 °C and add sulphanilamide and N-1-naphthyl-ethylenediamine dihydrochloride. The absorbance was noted at 540 nm. The results were demonstrated as nmol NO_2 kg^{-1} (leaf FM) s^{-1}.

2.4.6. Leaf Nitrogen, Phosphorus and Potassium Contents

The plant leaves were dried in an oven. After drying, they were powdered. The leaf powder (100 mg) was taken and transferred to a 50 mL Kjeldahl flask, to which 2 mL H_2SO_4 was added. The contents were heated on the Kjeldahl assembly for about 2 h, followed by cooling for about 15 minutes at room temperature. An amount of 0.5 mL of hydrogen peroxide (30%) was added, and the heating was repeated. The process of adding hydrogen peroxide drop by drop and heating was continued until the solution colour changed from black to light. The complete digested sample was taken and make the final volume with DDW. The N, P and K contents were determined from this digested material. Procedures of [20] and [21] were followed for estimating N and P contents, respectively. By using a flame-photometer [22], estimated leaf K content. The leaf's N, P and K contents were demonstrated as a percentage (%) on a dry weight basis.

2.5. Microscopical Studies

2.5.1. Scanning Electron Microscopy (SEM)

The fresh leaves were immediately immersed in a mixture of 2% paraformaldehyde, 2.5% glutaraldehyde and 0.1 M sodium cacodylate buffer (pH 7.3) for two hours. The samples were dried with different ethanol concentrations (50, 70, 80, 90 and 100%). The thoroughly dried leaves were cut into small pieces of about 2 cm^2. These pieces were then taken and fixed with gold-palladium, and stomatal aperture images were captured using SEM (JSM-6510LV, JEOL, Tokyo, Japan). Moreover, the leaves were dried and ground to powder. To estimate mineral nutrients accumulation, powdered samples were assessed through energy-dispersive X-ray spectroscopy (EDS).

2.5.2. Confocal Scanning Microscopy

The roots of the plants were immersed in the propidium iodide solution to study cell viability. Then roots were cleaned with DDW. The slides of the samples were made and observed in a confocal scanning microscope (Zeiss, LSM 780, Germany). The scanning microscopy was done only in samples of cultivars receiving sprays of water and 45 ppm S.

2.6. Yield and Quality Characteristics

Yield and quality attributes, namely, the pods of a plant, seeds per pod, 1000-seed weight, seed yield of a plant, oil content and oil yield of a plant, were evaluated at harvest.

2.7. Gas Chromatography-Mass Spectroscopy

The oil was analyzed by using a gas chromatography-mass spectroscope (GCMS-TQ8050 NX, Japan, in the Central Instrumentation Laboratory CUPB Ghudda, Bathinda, India). The GC conditions were set as follows: interface temperature: 250.00 °C, detector gain: 0.96 kV + 0.20 kV, solvent cut time: 4.50-min, detector gain mode: relative to the tuning result, threshold: 0. and ion source temperature: 230.00 °C. The compounds were identified via the standard mass spectrum database of NIST17R.lib and NIST17M2. lib. The GC-MS analysis was performed only in the oil of plants receiving spray of water and 45 ppm S.

2.8. Statistical Analysis

Through two-way analysis of variance (ANOVA) using SPSS 16.0 software (windows 11) for the analysis of experimental data. The differences in treatment means were checked by Tukey's Test at $p \leq 0.05$.

3. Results

The treatment´s effects and their interaction with cultivars were found to be significant on all parameters studied except the interaction effect on branches and leaves per plant, relative water content and seeds per pod. Among the foliar spray treatments, 45 ppm S gave the highest value, followed by 30 ppm S, whereas, among the cultivars, Nath Sona performed best and was followed by Rohini and Chutki on almost all the parameters studied.

3.1. Growth Characteristics

The growth traits were increased with the foliar spray of 45 ppm S in all three cultivars. The application of 45 ppm S enhanced the shoot length per plant by 13.67, 13.12 and 8.80%, branches per plant by 30, 25 and 23.07%, leaves per plant by 33.91, 32.11 and 27.72%, area per leaf by 30.52, 28.36 and 26.72%, leaf area of a plant by 29.52, 36 and 28.32%, fresh weight of a plant by 43.69, 14.76 and 19.19% and dry weight of a plant by 40.21, 35.65 and 30.96% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 1A–F and Figure 2A).

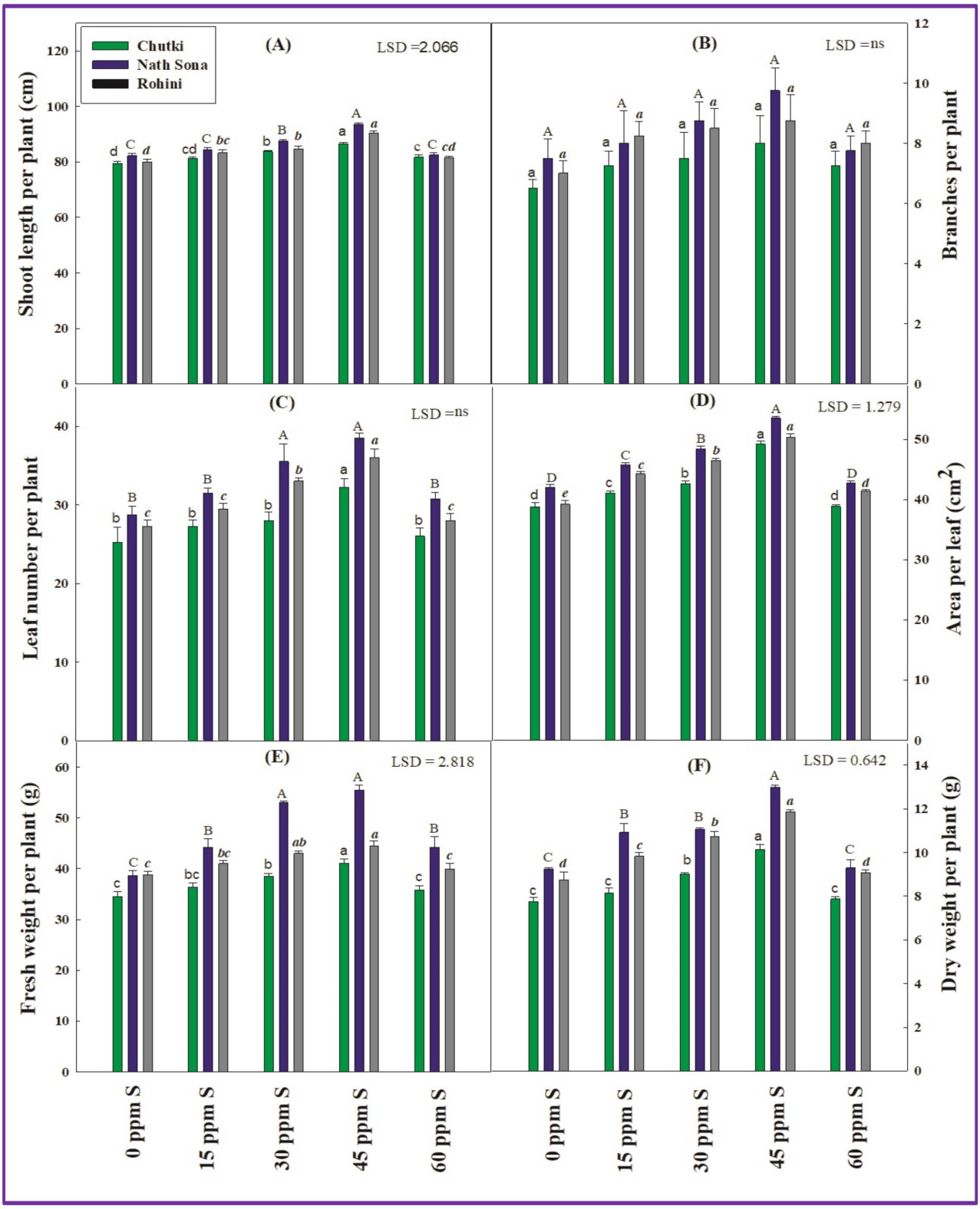

Figure 1. Effect of different doses of sulphur on (**A**) shoot length per plant, (**B**) branches per plant, (**C**) leaf number per plant, (**D**) area per leaf, (**E**) fresh weight per plant and (**F**) dry weight per plant of mustard cultivars Chutki, Nath Sona and Rohini. Data are treatments means and standard error (±SE) of each cultivar's four replicates (n = 4). The bar shows SE and different lower- and upper-case letters represent a significant difference in three cultivars among various treatments by Tukey's test at $p \leq 0.05$.

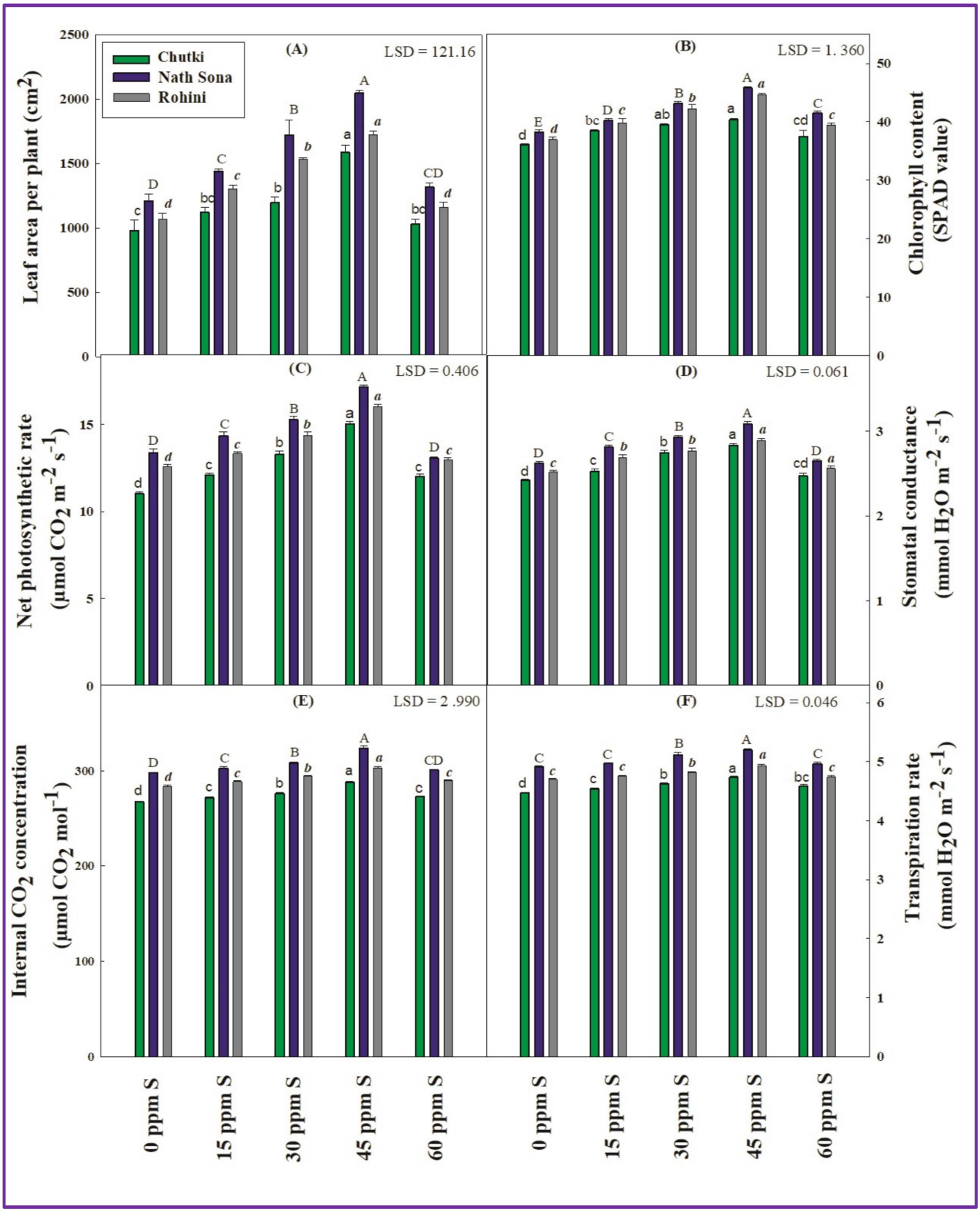

Figure 2. Effect of different doses of sulphur on (**A**) leaf area per plant, (**B**) chlorophyll content, (**C**) net photosynthetic rate, (**D**) stomatal conductance, (**E**) internal CO_2 concentration and (**F**) transpiration rate of mustard cultivars Chutki, Nath Sona and Rohini. Data are treatments means and standard error (±SE) of each cultivar's four replicates (n = 4). The bar shows SE and different lower- and upper-case letters represent a significant difference in three cultivars among various treatments by Tukey's test at $p \leq 0.05$.

3.2. Physio-Biochemical Parameters

3.2.1. Chlorophyll Content

The spray treatment of 45 ppm S enhanced SPAD value by 19.75, 20.79 and 11.74% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 2B).

3.2.2. Gas Exchange Parameters

The foliar spray of 45 ppm S gave the maximum values for gas exchange parameters. The application of 45 ppm S increased P_N by 28.27, 27.44 and 36.29%, Ci by 13, 6.78 and 7.47%, *gs* by 17.55, 15.13 and 16.94% and E by 5.70, 4.89 and 6.05% in cultivars Nath Sona, Rohini and Chutki respectively over their respective water-treated plants (Figure 2C–F).

3.2.3. Relative Water Content

The foliar spray of 45 ppm S enhanced RWC by 11.89, 12.92 and 11.49% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 3A).

3.2.4. Carbonic Anhydrase and Nitrate Reductase Activities

Among the different levels of S spray treatment, the foliar spray of 45 ppm S improved CA activity by 8.95, 11.57 and 23.48% and NR activity by 8.05, 6.26 and 6.66% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 3B,C).

3.2.5. Mineral Nutrient Contents

The spray treatment of 45 ppm S gave the highest value for N, P and K contents. The application of 45 ppm S enhanced N content by 8.94, 8.01 and 11.26%, P content by 13.27, 11.76 and 10.13% and K content by 7.51, 10.16 and 12.67% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 3D–F).

3.3. Yield and Quality Parameters

The spray treatment of 45 ppm S proved influential for pod number, 1000-seed weight, seed yield, oil content and oil yield of the plant and was equaled by that of 30 ppm S for seeds per pod. The spray treatment 45 ppm S improved pods of a plant by 15.23, 12.12 and 10.80%, seeds of a pod by 32.18, 28.57 and 34.61%, 1000-seed weight by 8.69, 8.42 and 7.82%, seed yield of a plant by 7.54, 3.89 and 4.91%, oil content by 48.70, 46.31 and 43.15% and oil yield of a plant by 24.56, 23.93 and 22.35% in cultivar Nath Sona, Rohini and Chutki, respectively, over their respective water-treated plants (Figure 4A–F).

3.4. Microscopical Studies

3.4.1. Scanning Electron Microscopy

The middle part (around the midrib) of the leaf (about 2 cm^2) of the non-treated (water sprayed) and treated plants were scanned under an electron microscope. The foliar application of S indicates a reduction of the stomatal aperture over the water-spray treatment. The spray treatment of 60 ppm S was shown to effectively reduce the stomatal aperture in our study (Figure 5A–E); however, it requires further investigation to validate the role of foliar applied S in regulating stomatal behaviour.

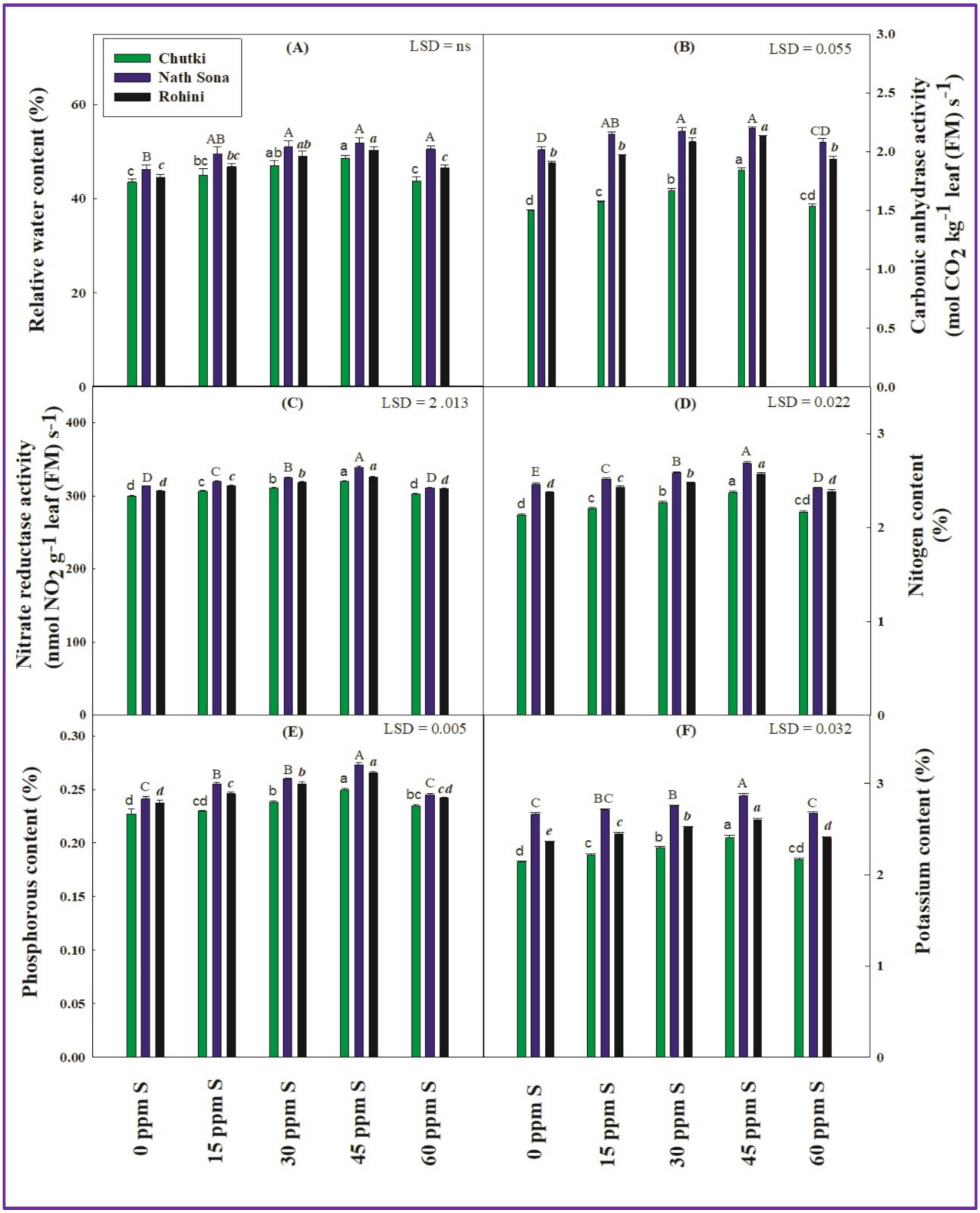

Figure 3. Effect of different doses of sulphur on (**A**) Relative water content, (**B**) Carbonic anhydrase activity, (**C**) Nitrate reductase activity, (**D**) Nitrogen content, (**E**) Phosphorous content and (**F**) Potassium content of mustard cultivars Chutki, Nath Sona and Rohini. Data are treatments means and standard error (± SE) of each cultivar's four replicates (n = 4). The bar shows SE and different lower- and upper-case letters represent a significant difference in three cultivars among various treatments by Tukey's test at $p \leq 0.05$.

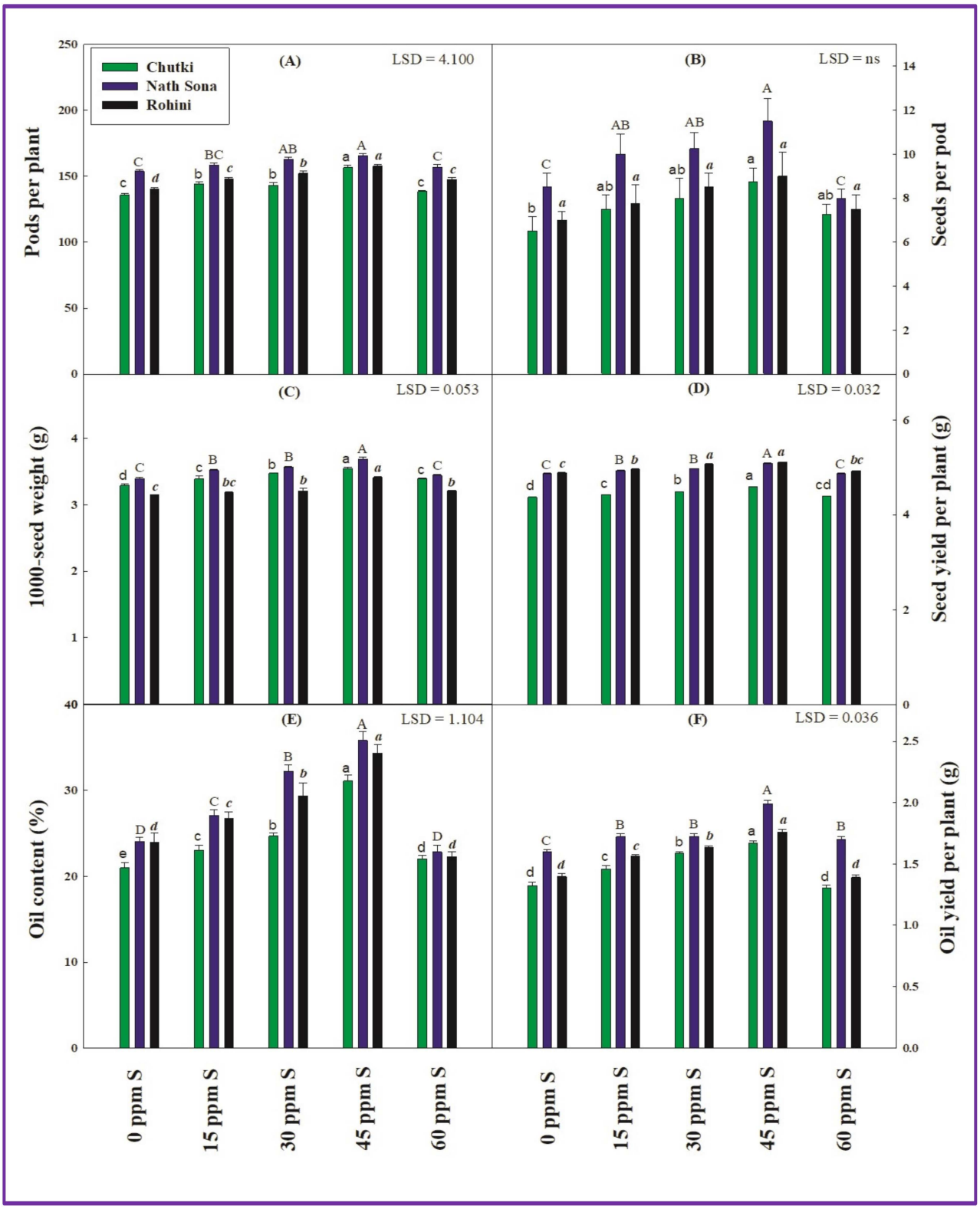

Figure 4. Effect of different doses of sulphur on (**A**) pods per plant, (**B**) seeds per pod, (**C**) 1000-seed weight, (**D**) seed yield per plant, (**E**) oil content and (**F**) oil yield per plant of mustard cultivars Chutki, Nath Sona and Rohini. Data are treatments means and standard error (±SE) of each cultivar's four replicates (n = 4). The bar shows SE and different lower- and upper-case letters represent a significant difference in three cultivars among various treatments by Tukey's test at $p \leq 0.05$.

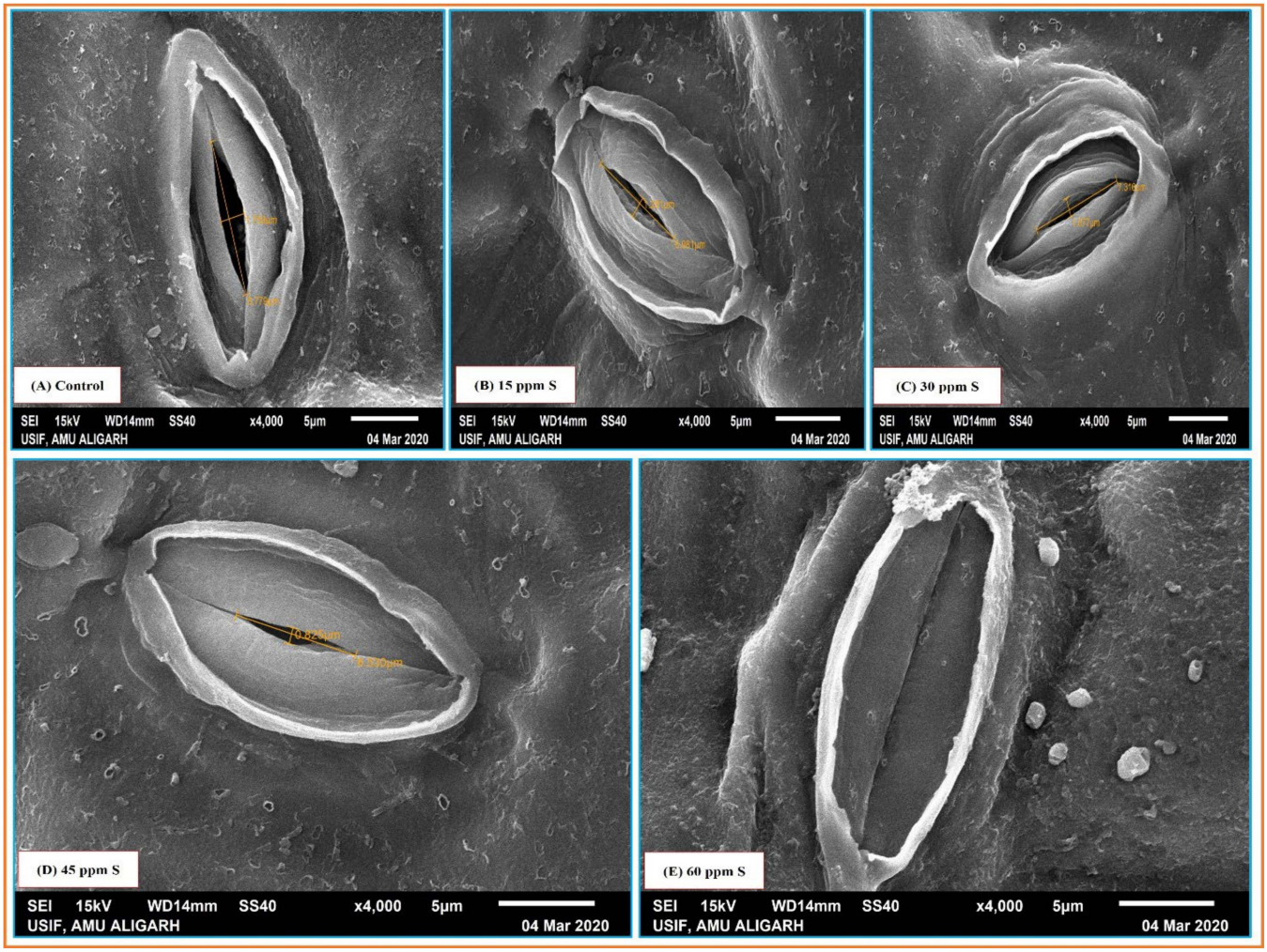

Figure 5. Scanning electron microscope images (at 4000× magnification) of stomatal response of mustard leaves at 90 DAS: (**A**) water-spray treatment and (**B**–**E**) 15, 30, 45 and 60 ppm S.

3.4.2. Confocal Scanning Microscopy

Propidium iodide (PI) is a red-fluorescent cell and nucleic acid staining dye. It penetrates the cell membrane of dead cells. After penetration, it reacts with nucleic acid to indicate dead cells. Compared with water-spray treatment, the spray treatment of 45 ppm S reduced the intensity of cell death as there were comparatively fewer illuminating fluorescent nuclei (Figure 6A–D).

3.5. GC-MS Analysis of Oil

Figure 7A (water-spray treatment) and Figure 7B (45 ppm S spray treatment) show gas chromatograms (A and B) of the oil content of cultivar Nath Sona (best performer). They showed the presence of many compounds with different retention times and area%. There were 36 compounds in the oil content of plants receiving water spray treatment and 44 compounds found in the oil content of plants sprayed with 45 ppm S. Eighteen compounds were common in both treatments (Table 1). Some compounds were found to be present only in the oil content of plants receiving water-spray treatment are nonanal, benzyl benzoate, 1-hexadecanol, linoleic acid-ethyl ester, ethyl oleate, 1-phenyl-1,3,8-triazaspiro-[4.5]-decan-4-one, 6aS,10aS-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyl), theophylline, methenolone acetate and 3-beta-hydroxy-5-cholen-24-oic acid. On the other hand, some important compounds were found to be present only in the oil content of plants receiving 45 ppm S spray: heneicosane, phthalic acid-octadecyl-pentafluoro-phenyl-ester, tetracontane, tetratetracontane, behenic alcohol, dotriacontane, beta-sitosterol acetate, nonadecane,

pentadecane, octacosane, tetracosane, 1-hentetracontanol and decane-1-iodo. These compounds are well known for their antioxidant, antimicrobial, anti-inflammatory and statin activity.

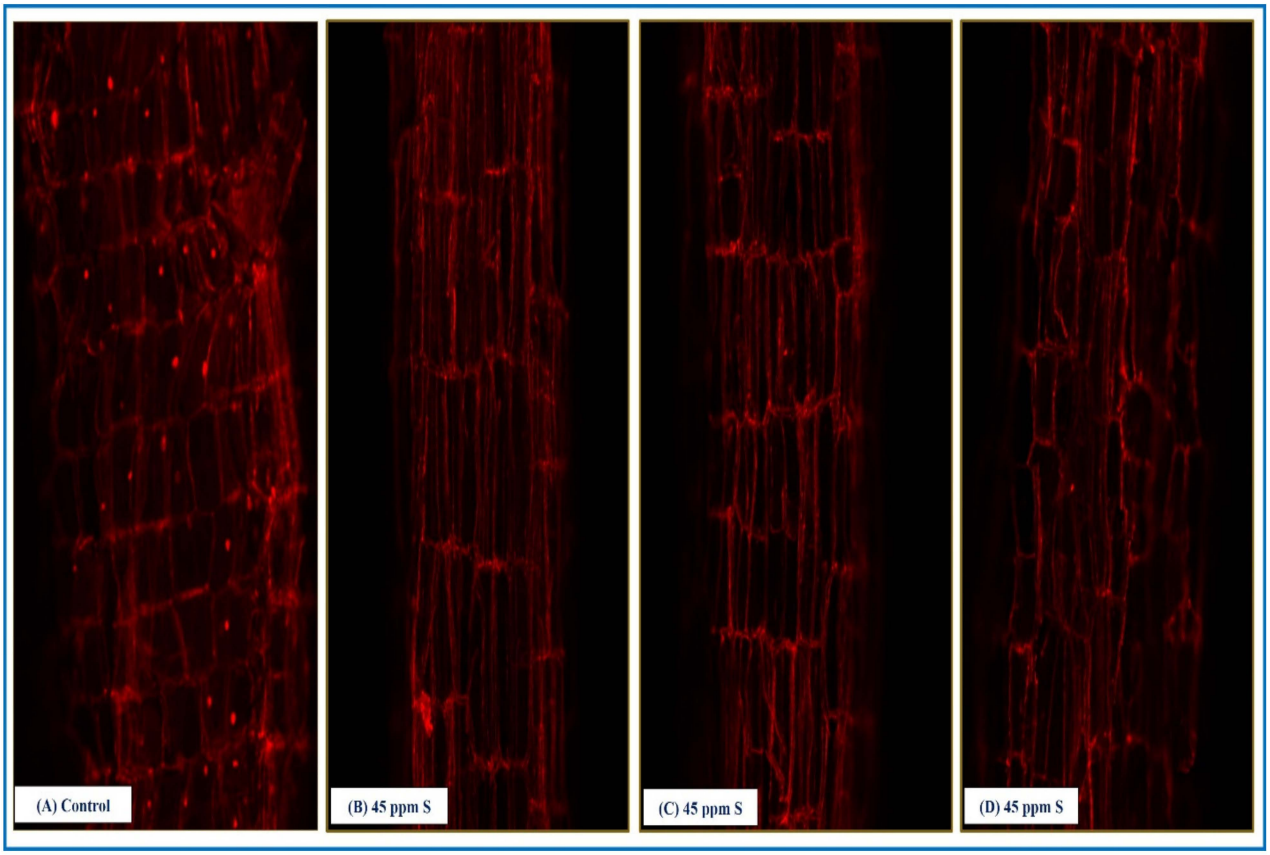

Figure 6. Confocal microscopic images showing cell viability in root cells of mustard cultivars Chutki, Nath Sona and Rohini at 90 DAS: (**A**) water-spray treatment, (**B**–**D**) 45 ppm S.

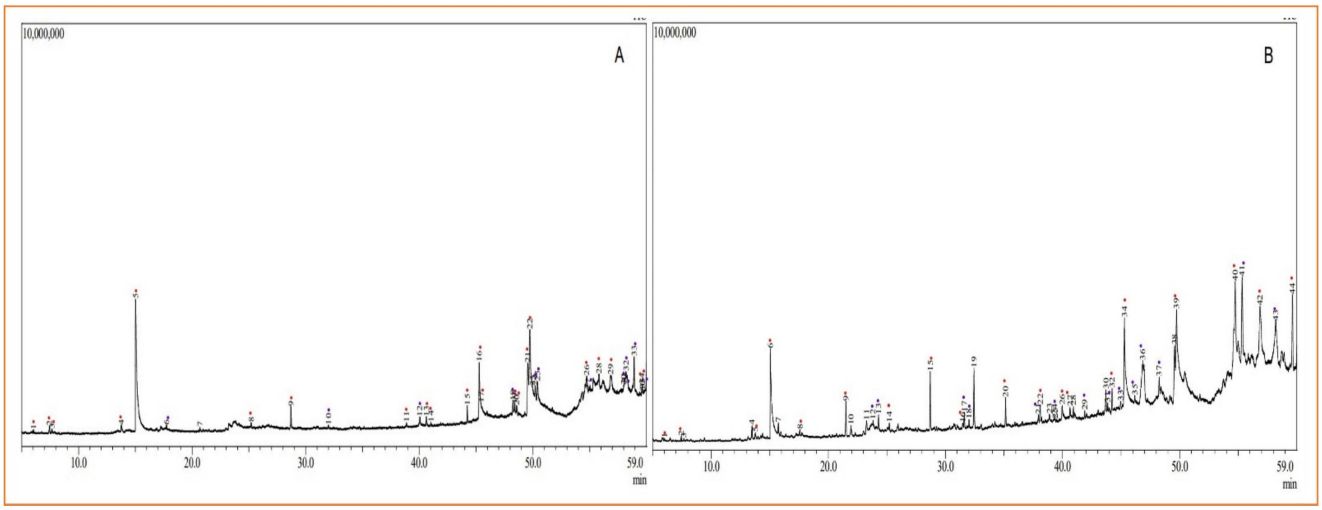

Figure 7. GC-MS analysis showing chemical compounds in the oil content of mustard cultivar Nath Sona receiving water-spray treatment (**A**) and 45 ppm S spray treatment (**B**). Red dots in the chromatogram (**A**,**B**) indicate the common compounds enlisted in Table 1. Blue dots indicate the compounds present only in the oil of the water-treated plants and the oil of 45 ppm in the S-treated plants.

Table 1. Similar compounds identified in the oil content of mustard cultivar Nath Sona receiving 0 (water) and the 45 ppm S treatment by GC-MS analysis.

S. No.	Retention Time	Name of the Compound	Peak Area%	
			0 (water)	45 ppm S
1	6.012	Hexanal	0.27	0.04
2	7.440	2-Propanol, 1,1′-oxybis	0.46	0.12
3	13.754	Phenol	1.05	0.19
4	15.043	2-Pyrrolidinone, 1-methyl	26.87	4.46
5	15.729	Dodecane, 4,6-dimethyl	0.42	0.34
6	17.575	Tetradecane	2.14	0.13
7	25.204	Tridecane	0.41	0.16
8	28.704	Tetradecane	2.14	1.60
9	31.479	Decane, 1-iodo	0.32	0.11
10	39.972	Pentadecanoic acid	0.34	0.41
11	40.637	2-Propanol, 1-chloro-, phosphate	0.94	0.25
12	44.228	Hexadecanoic acid, methyl ester	1.95	0.77
13	45.282	n-Hexadecanoic acid	9.65	4.70
14	49.563	9,12-Octadecadienoic acid (Z, Z)	0.61	3.03
15	49.733	9-Octadecenoic acid, 1,2,3-propanetriyl ester	0.89	12.93
16	54.744	11-Dehydrocorticosterone	4.30	13.69
17	56.859	Tetrapentacontane	3.76	16.85
18	59.630	Bis (2-ethylhexyl) phthalate	1.17	3.63

4. Discussion

Better growth, photosynthesis, and yield of oilseed crops are achievable only when crops receive the required amount of S in addition to other essential mineral nutrients. Sulphur is a constituent of the important metabolites of plants, including iron-S clusters, amino acids, lipids and polysaccharides, disulphides, peptides, co-factors, glucosinolates and precursors of plant hormones [1]. The S-containing metabolites control numerous physiological and biochemical processes in plants. In addition, foliar feeding of S has been known to influence the growth, yield and quality parameters of oilseed crops [23,24]. The present study is designed to evaluate the responses of three cultivars of mustard to the foliar spray of S.

4.1. Growth and Biomass of Plants

The results (Figure 1A–F and Figure 2A) showed that the foliar spray of S up to 45 ppm S enhanced the growth characteristics such as shoot length, branches and leaf number of a plant, area of a leaf, leaf area of a plant, fresh and dry weight of a plant over the course of water-spray treatments. The enhancing effect of S on shoot length, branch number, leaf number, unit leaf area and entire leaf area of a plant may be ascribed to the roles of S in plants. The S-containing compounds might entail cell enlargement, cell division, and tissue and organ formation leading to the enhancement of growth attributes [1,25]. Our results agreed with the studies on groundnut [26], rapeseed [27], and canola [28,29]. They revealed that the S fertilization improved the growth traits of oilseed crops. The enhancement in these growth traits might have culminated in the production of fresh mass, hence the higher values for the fresh weight of the plant. The improved fresh weight of plants would naturally lead to higher dry weight of plants [30,31].

4.2. Photosynthesis and Other Parameters

The foliar spray of 45 ppm S solution improved the leaf chlorophyll content, P_N, g_s, C_i, E, and RWC during the water treatment (Figure 2B–F and Figure 3A). The improvements in photosynthetic pigment due to the application of S may be explained as follows. The spray of S increases the S content in leaf tissues, leading to a decrease in cell-sap pH. The S-mediated reduction in pH of cell sap makes iron available to plant cells for porphyrin

biosynthesis. Porphyrin is an intermediate in the biosynthesis of chlorophyll. Moreover, S enhances the assimilation of nutrients like N and magnesium, which are associated with the stability of chlorophyll structure [28,32,33]. Thus, S directly or indirectly improves chlorophyll content. The S-mediated improvement in chlorophyll content of mustard has also been investigated by [4,34]. The observed improvements in P_N, g_s, C_i and E due to foliar spray of S may be ascribed to the role of S in the biosynthesis of photosynthetic pigments and components of photosynthetic apparatus and activity of rubisco enzyme, among others. The increase in these metabolites and enzyme activity would have improved photosynthetic parameters hence their higher values. These results are similar to those studies [4,6,35] on mustard and [36] on rice. They investigated that the fertilization of S improved the photosynthetic efficiency of plants. The increase in RWC in S-sprayed plants over water-sprayed plants can be ascribed to the uptake and retention of water content in plant tissues and the metabolic balance of cells resulting from the S-containing metabolites mediated redox homeostasis, membrane stability and metabolic balance of the cell.

Our results are validated by those of a rapeseed study [37]. In our study, CA and NR enhanced activities were noticed due to the foliar application of S over water-spray treatment (Figure 3B,C). NR is a key enzyme that catalyzes the first step in reducing nitrate to nitrite for the acquisition of N in plants. This reaction is crucial for producing proteins in plants [38]. CA is an important enzyme for photosynthesis and plays a role in carbon fixation, i.e., CO_2 to HCO_3^-. It is also involved in stomatal closure in plant leaves [39]. The improvements in CA and NR activities due to the foliar application of S may be related to enhancement in enzyme concentrations resulting from S-mediated improved synthesis of proteins. Our findings are validated by studies on groundnut [40] and wheat [3].

4.3. Mineral Nutrient Contents

In this study, the improvements in leaf N, P and K contents due to the foliar spray of S (Figure 3C–F) can be attributed to S-mediated enhancement in the uptake and assimilation of mineral nutrients. Sulphur improves the N-use efficiency of plants [13], which would have, in turn, influenced the absorption of other nutrients like P and K hence higher values for leaf N, P and K contents of S-sprayed plants. Moreover, S application influenced the accumulation of mineral nutrient content in leaves (Figure 8). An improvement in leaf nutrient contents due to the application of S has been revealed by [41,42] in the context of wheat and sesame, respectively.

4.4. Microscopical Studies

In our study, the observed reduction in stomatal width with the increasing levels of S in the spray over the water-spray treatment (Figure 5A–E) may be due to the accumulation of sulphate that would have induced abscisic acid biosynthesis responsible for the closing of stomata. Moreover, the exogenous S application could directly regulate the R-Type anion channel Quick Anion Channel 1 (QUAC1), which is involved in inducing stomatal closure. In addition, the S is the precursor molecule in hydrogen sulfide (H_2S) synthesis, which interacts with the ABA signaling cascade to regulate stomatal closure [43]. The effect of S application in regulating the stomatal behaviour was also reported by [44] working on mustard. However, it needs more detailed analysis to confirm how the high concentration of S application reduced the stomatal aperture.

Further, in our study, the root cell viability of S-treated plants was more than the water-spray treatment (Figure 6). Sulphur is a constituent of many cellular metabolites involved in maintaining cellular homeostasis and regulating cell division. The exogenous supply of S (stored as sulfate in the leaf vacuoles) can be remobilized through the various sulfate transporters to different parts of the plant at different developmental phases leading to the improvement in the longevity of cells, tissues and organs. Moreover, it was also suggested that the application of S promotes the biosynthesis of auxin, which plays a key role in root development [1,7] hence the enhancement in the viability of root cells.

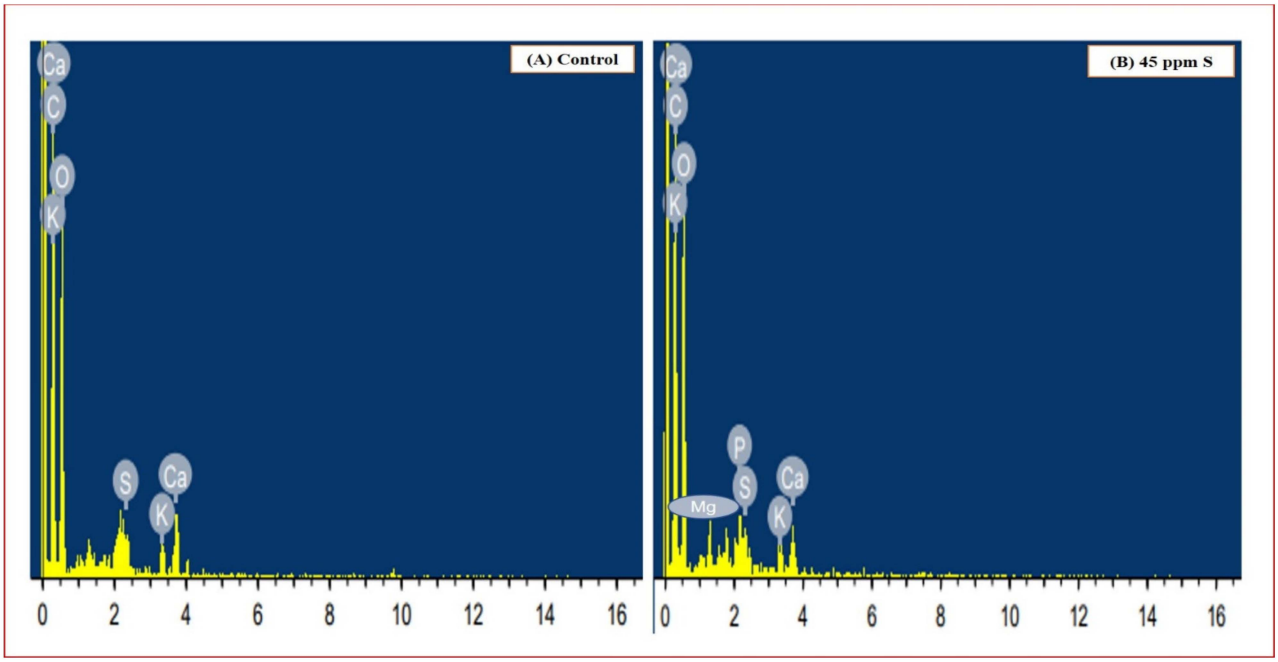

Figure 8. Mineral nutrient accumulation detected by energy dispersive X-ray spectroscopy (EDS) in mustard leaf at 90 DAS (**A**) water spray-treatment (**B**) 45 ppm S.

4.5. Yield and Quality Attributes

The foliar spray of 45 ppm S solution improved a plant pods, seeds of a pod, 1000-seed weight, seed yield, oil content and oil yield of a plant over the water-spray treatment (Figure 4A–F). The improvements in pods of a plant, seeds of a pod and 1000-seed weight may be the result of improved growth and physio-biochemical characteristics of S-treated plants. These yield parameters would have attributed to seed yield per plant hence higher values for seed yield of S-sprayed plants. The improvements in leaf area and photosynthetic rate by S application seem responsible for increased yield characteristics. The improved leaf area of treated plants shows efficient carbon assimilation and enables them to produce a higher amount of photosynthates, which positively affects yield attributes. The increase in seed yield might be due to the role of S in enhancing growth traits, additional synthesis of chlorophyll content, higher photosynthetic efficiency and cell division and more translocation of photosynthates towards seeds. Moreover, with the S fertilization, tissue differentiation from somatic meristematic to reproductive and developmental activity has increased, resulting in more flowers and pods and a higher seed yield [2,23].

The improvements in oil content may be directly related to the role of S in oil synthesis in plants [45,46]. Sulphur fertilization improves the process of oil composition and acetyl CoA content. The improvement in yield and quality parameters may be due to the improved growth and photosynthetic parameters and nutrient contents of plants. Moreover, S promotes the formation of saturated fatty acids and oil storage organs [29], and the S stored in the form of sulphate in the leaves of oilseed crops also contributes to seed filling during the reproductive stage, leading to improved seed yield and oil content. The enhanced seed yield, as well as oil content, would naturally result in higher oil yield. In addition, the enhancement in the productivity of mustard by the foliar application of S is also verified by PCA analysis (Figure 9). Our results resemble with the studies on maize [47], lettuce [48] and mustard [49,50].

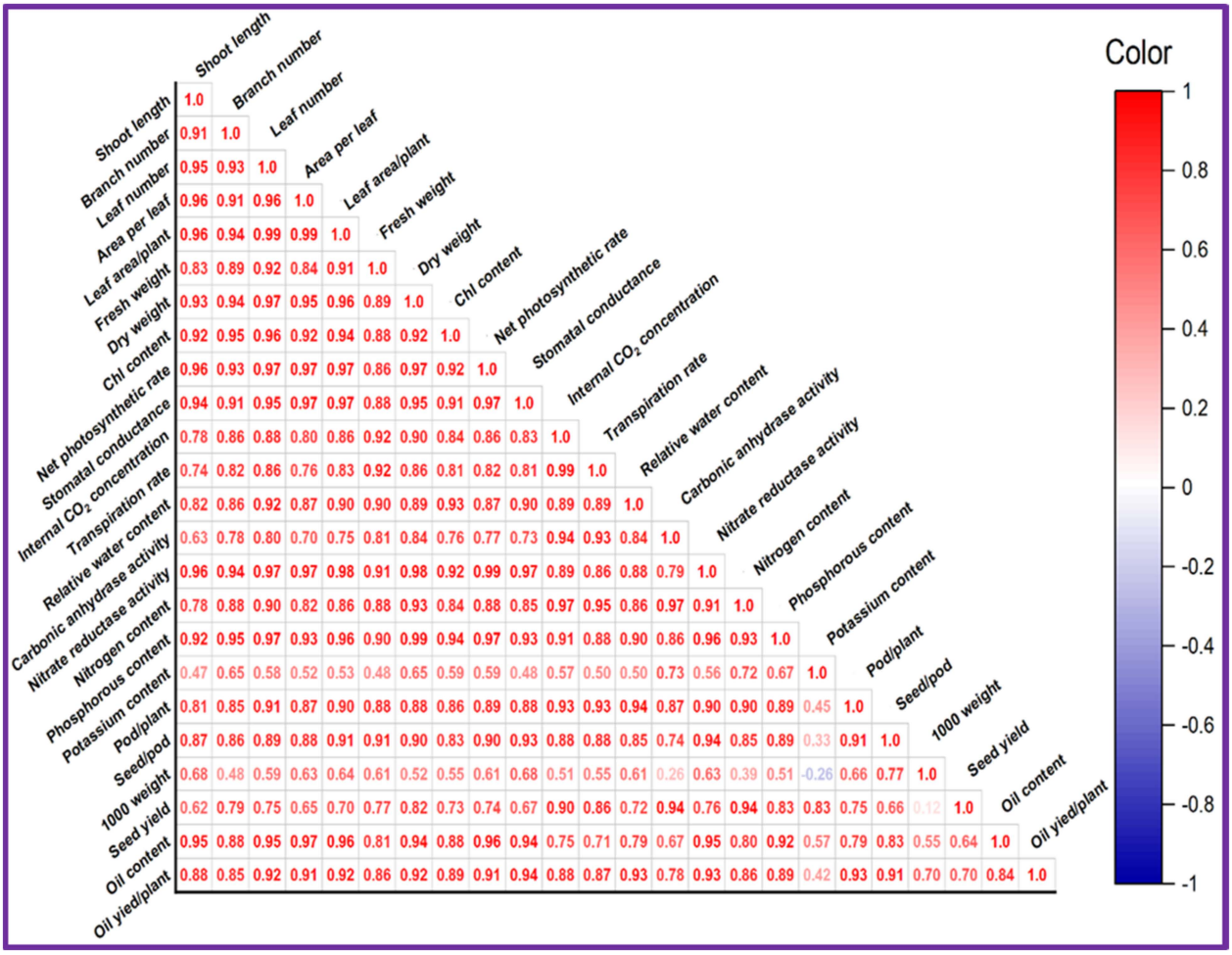

Figure 9. Principal component analysis of various growth, physio-biochemical and yield parameters.

The superior performance of the Nath Sona cultivar under the foliar application of 45 ppm S could be due to its high capacity to accumulate and utilize S, leading to better growth, metabolic processes and productivity. The decrease in physio-biochemical and yield parameters resulting from the highest concentration of S spray (60 ppm S) may be due to its toxic effect. This study observed that the effect of foliar spray of different concentrations of S is dose-dependent and improved all studied parameters of the three cultivars of mustard in a similar trend.

5. Conclusions

Sulphur influences growth, photosynthesis, enzymatic activities, mineral nutrient contents, yield and quality of oilseed crops, including mustard. The present study reveals that foliar application of S improves growth, biomass, physiological processes and productivity of three cultivars of mustard. Keeping the results of this study in mind, it may be concluded that the genetic potential of cultivars of mustard, particularly Nath Sona, can be exploited by growing them with the recommended basal dose of nutrients supplemented with two sprays of 45 ppm S at appropriate growth stages. More studies are required to investigate the effect of various sources of inorganic S on crop performance. Moreover, more research is needed to identify and localize the S transporters involved in the absorption of S in leaves and its subsequent transportation to the other parts of plants.

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F.M. designed and supervised the experiment and drafted the MS. All authors have read and agreed to the published version of the manuscript.

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References

1. Shah, S.H.; Islam, S.; Mohammad, F. Sulphur as a dynamic mineral element for plants: A review. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 2118–2143. [CrossRef]
2. Siddiqui, M.H.; Mohammad, F.; Khan, M.M.A.; Al-Whaibi, M.H. Cumulative effect of nitrogen and sulphur on *Brassica juncea* L. genotypes under NaCl stress. *Protoplasma* **2012**, *249*, 139–153. [CrossRef] [PubMed]
3. Khan, M.I.R.; Nazir, F.; Asgher, M.; Per, T.S.; Khan, N.A. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *J. Plant Physiol.* **2015**, *173*, 9–18. [CrossRef] [PubMed]
4. Fatma, M.; Asgher, M.; Masood, A.; Khan, N.A. Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. *Environ. Exp. Bot.* **2014**, *107*, 55–63. [CrossRef]
5. Meena, R.S.; Kumar, S.; Bohra, J.S.; Lal, R.; Yadav, G.S.; Pandey, A. Response of alley cropping-grown sesame to lime and sulphur on yield and available nutrient status in an acidic soil of Eastern India. *Energy Ecol. Environ.* **2019**, *4*, 65–74. [CrossRef]
6. Bano, K.; Kumar, B.; Alyemeni, M.N.; Ahmad, P. Protective mechanisms of sulfur against arsenic phytotoxicity in *Brassica napus* by regulating thiol biosynthesis, sulfur-assimilation, photosynthesis, and antioxidant response. *Plant Physiol. Biochem.* **2022**, *188*, 1–11. [CrossRef]
7. Narayan, O.P.; Kumar, P.; Yadav, B.; Dua, M.; Johri, A.K. Sulfur nutrition and its role in plant growth and development. *Plant Signal. Behav.* **2022**, 2030082. [CrossRef]
8. Islam, S.; Mohammad, F. Modulation of growth, photosynthetic efficiency, leaf biochemistry, cell viability and yield of Indian mustard by the application of trehalose. *Sci. Hortic.* **2021**, *290*, 110527. [CrossRef]
9. Ahmad, P.; Ahanger, M.A.; Alyemeni, M.N.; Wijaya, L.; Egamberdieva, D.; Bhardwaj, R.; Ashraf, M. Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) Czern & Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. *J. Plant Interact.* **2017**, *12*, 429–437.
10. Islam, S.; Parrey, Z.A.; Shah, S.H.; Mohammad, F. Glycine betaine mediated changes in growth, photosynthetic efficiency, antioxidant system, yield and quality of mustard. *Sci. Hortic.* **2021**, *285*, 110170. [CrossRef]
11. Sharma, R.; Bhardwaj, R.; Thukral, A.K.; Al-Huqail, A.A.; Siddiqui, M.H.; Ahmad, P. Oxidative stress mitigation and initiation of antioxidant and osmoprotectant responses mediated by ascorbic acid in *Brassica juncea* L. subjected to copper (II) stress. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109436. [CrossRef]
12. Kohli, S.K.; Bali, S.; Tejpal, R.; Bhalla, V.; Verma, V.; Bhardwaj, R.; Ahmad, P. In-situ localization and biochemical analysis of bio-molecules reveals Pb-stress amelioration in *Brassica juncea* L. by co-application of 24-Epibrassinolide and Salicylic Acid. *Sci. Rep.* **2019**, *9*, 3524. [CrossRef]
13. Poisson, E.; Trouverie, J.; Brunel-Muguet, S.; Akmouche, Y.; Pontet, C.; Pinochet, X.; Avice, J.C. Seed yield components and seed quality of oilseed rape are impacted by sulfur fertilization and its interactions with nitrogen fertilization. *Front. Plant Sci.* **2019**, *10*, 458. [CrossRef]
14. Shah, S.H.; Islam, S.; Parrey, Z.A.; Mohammad, F. Role of exogenously applied plant growth regulators in growth and development of edible oilseed crops under variable environmental conditions: A review. *J Soil Sci. Plant Nutr.* **2021**, *21*, 3284–3308. [CrossRef]
15. Reddi, M.V.; Reddy, P.S. Commercial crops (oilseed crops). In *Handbook of Agriculture*, 5th ed.; Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, Krishi Anusandhan Bhavan: New Delhi, India, 2003; pp. 921–1048s.
16. Zhang, X.; Kang, J.; Pang, H.; Niu, L.; Lv, J. Sulfur mediated improved thiol metabolism, antioxidant enzymes system and reduced chromium accumulation in oilseed rape (*Brassica napus* L.) shoots. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35492–35500. [CrossRef]

17. Jones, M.M.; Turner, N.C. Osmotic adjustment in leaves of sorghum in response to water deficits. *Plant Physiol.* **1978**, *61*, 122–126. [CrossRef]
18. Dwivedi, R.S.; Randhawa, N.S. Evaluation of a rapid test for the hidden hunger of zinc in plants. *Plant Soil* **1974**, *40*, 445–451. [CrossRef]
19. Jaworski, E.G. Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1274–1279. [CrossRef]
20. Lindner, R.C. Rapid analytical methods for some of the more common inorganic constituents of plant tissues. *Plant Physiol.* **1944**, *19*, 76. [CrossRef]
21. Fiske, C.H.; Subbarow, Y. The colorimetric determination of phosphorus. *Biochem. J.* **1925**, *66*, 375–400. [CrossRef]
22. Hald, P.M. The flame photometer for the measurement of sodium and potassium in biological materials. *J. Biochem.* **1946**, *163*, 499–510. [CrossRef]
23. Raza, M.A.; Feng, L.Y.; Manaf, A.; Wasaya, A.; Ansar, M.; Hussain, A.; Yang, W. Sulphur application increases seed yield and oil content in sesame seeds under rainfed conditions. *Field Crops Res.* **2018**, *218*, 51–58. [CrossRef]
24. Sikorska, A.; Gugała, M.; Zarzecka, K. The response of different kinds of rapeseed cultivars to foliar application of nitrogen, sulphur and boron. *Sci. Rep.* **2021**, *11*, 21102. [CrossRef]
25. Scherer, H.W. Sulphur in crop production. *Eur. J. Agron.* **2001**, *14*, 81–111. [CrossRef]
26. Dileep, D.; Singh, V.; Tiwari, D.; George, G.S.; Swathi, P. Effect of variety and sulphur on growth and yield of groundnut (*Arachis hypogaea* L.). *Biol. Forum Int. J.* **2021**, *13*, 475–478.
27. Kaur, M.; Kumar, S.; Kaur, A. Effect of foliar application of nitrogen, phosphorus and sulphur on growth and yield of Gobhi Sarson (*Brassica napus* L.) in central Punjab. *J. Oilseed Brassica* **2019**, *10*, 47–50.
28. Ur Rehman, H.; Iqbal, Q.; Farooq, M.; Wahid, A.; Afzal, I.; Basra, S.M. Sulphur application improves the growth, seed yield and oil quality of canola. *Acta Physiol. Plant.* **2013**, *35*, 2999–3006. [CrossRef]
29. Waraich, E.A.; Hussain, A.; Ahmad, Z.; Ahmad, M.; Barutçular, C. Foliar application of sulfur improved growth, yield and physiological attributes of canola (*Brassica napus* L.) under heat stress conditions. *J. Plant Nutr.* **2022**, *45*, 369–379. [CrossRef]
30. Ali, M.M.; Waleed Shafique, M.; Gull, S.; Afzal Naveed, W.; Javed, T.; Yousef, A.F.; Mauro, R.P. Alleviation of Heat Stress in Tomato by Exogenous Application of Sulfur. *Horticulturae* **2021**, *7*, 21. [CrossRef]
31. Hussain, S.J.; Masood, A.; Anjum, N.A.; Khan, N.A. Sulfur-mediated control of salinity impact on photosynthesis and growth in mungbean cultivars screened for salt tolerance involves glutathione and proline metabolism, and glucose sensitivity. *Acta Physiol. Plant.* **2019**, *41*, 129. [CrossRef]
32. Kumawat, R.N.; Rathore, P.S.; Nathawat, N.S.; Mahatma, M. Effect of sulfur and iron on enzymatic activity and chlorophyll content of mungbean. *J. Plant Nutr.* **2006**, *29*, 1451–1467. [CrossRef]
33. Skudra, I.; Ruza, A. Effect of nitrogen and sulphur fertilization on chlorophyll content in winter wheat. *Rural Sustain. Res.* **2017**, *37*, 29–37. [CrossRef]
34. Anjum, N.A.; Umar, S.; Ahmad, A.; Iqbal, M.; Khan, N.A. Sulphur protects mustard (*Brassica campestris* L.) from cadmium toxicity by improving leaf ascorbate and glutathione. *Plant Growth Regul.* **2008**, *54*, 271–279. [CrossRef]
35. Heikal, Y.M.; El-Esawi, M.A.; Galilah, D.A. Morpho-anatomical, biochemical and molecular genetic responses of canola (*Brassica napus* L.) to sulphur application. *Environ. Exp. Bot.* **2022**, *194*, 104739. [CrossRef]
36. Liu, J.; Hou, H.; Zhao, L.; Sun, Z.; Li, H. Protective effect of foliar application of sulfur on photosynthesis and antioxidative defense system of rice under the stress of Cd. *Sci. Total Environ.* **2020**, *710*, 136230. [CrossRef]
37. Namvar, A.; Khandan, T. Inoculation of rapeseed under different rates of inorganic nitrogen and sulfur fertilizer: Impact on water relations, cell membrane stability, chlorophyll content and yield. *Arch. Agron. Soil Sci.* **2015**, *61*, 1137–1149. [CrossRef]
38. Rehman, H.U.; Alharby, H.F.; Al-Zahrani, H.S.; Bamagoos, A.A.; Alsulami, N.B.; Alabdallah, N.M.; Wakeel, A. Enriching Urea with Nitrogen Inhibitors Improves Growth, N Uptake and Seed Yield in Quinoa (*Chenopodium quinoa* Willd) Affecting Photochemical Efficiency and Nitrate Reductase Activity. *Plants* **2022**, *11*, 371. [CrossRef]
39. DiMario, R.J.; Giuliani, R.; Ubierna, N.; Slack, A.D.; Cousins, A.B.; Studer, A.J. Lack of leaf carbonic anhydrase activity eliminates the C4 carbon-concentrating mechanism requiring direct diffusion of CO2 into bundle sheath cells. *Plant Cell Environ.* **2022**, *45*, 1382–1397. [CrossRef]
40. Jamal, A.; Fazli, I.S.; Ahmad, S.; Kim, K.T.; Oh, D.G.; Abdin, M.Z. Effect of sulfur on nitrate reductase and ATP sulfurylase activities in groundnut (*Arachis hypogea* L.). *J. Plant Biol.* **2006**, *49*, 513–517. [CrossRef]
41. Kulczycki, G.; Sacała, E.; Chohura, P.; Załuska, J. Maize and Wheat Response to Drought Stress under Varied Sulphur Fertilisation. *Agronomy* **2022**, *12*, 1076. [CrossRef]
42. Salvagiotti, F.; Castellarín, J.M.; Miralles, D.J.; Pedrol, H.M. Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. *Field Crops Res.* **2009**, *113*, 170–177. [CrossRef]
43. Ren, Z.; Wang, R.Y.; Huang, X.Y.; Wang, Y. Sulfur Compounds in Regulation of Stomatal Movement. *Front. Plant Sci.* **2022**, 495. [CrossRef] [PubMed]
44. Jahan, B.; AlAjmi, M.F.; Rehman, M.T.; Khan, N.A. Treatment of nitric oxide supplemented with nitrogen and sulfur regulates photosynthetic performance and stomatal behavior in mustard under salt stress. *Physiol. Plant* **2020**, *168*, 490–510. [PubMed]
45. Mondal, S.; Pramanik, K.; Panda, D.; Dutta, D.; Karmakar, S.; Bose, B. Sulfur in Seeds: An Overview. *Plants* **2022**, *11*, 450. [CrossRef]

46. Aarabi, F.; Rakpenthai, A.; Barahimipour, R.; Gorka, M.; Alseekh, S.; Zhang, Y.; Hoefgen, R. Sulfur deficiency-induced genes affect seed protein accumulation and composition under sulfate deprivation. *Plant Physiol.* **2021**, *187*, 2419–2434. [CrossRef]
47. Ma, Y.; Zhang, H.; Xue, Y.; Gao, Y.; Qian, X.; Dai, H.; Li, Z. Effect of sulfur fertilizer on summer maize grain yield and soil water utilization under different irrigation patterns from anthesis to maturity. *Agric. Water Manag.* **2021**, *250*, 106828. [CrossRef]
48. Muscolo, A.; Marra, F.; Canino, F.; Maffia, A.; Mallamaci, C.; Russo, M. Growth, nutritional quality and antioxidant capacity of lettuce grown on two different soils with sulphur-based fertilizer, organic and chemical fertilizers. *Sci. Hortic.* **2022**, *305*, 111421. [CrossRef]
49. Ray, K.; Sengupta, K.; Pal, A.K.; Banerjee, H. Effects of sulphur fertilization on yield, S uptake and quality of Indian mustard under varied irrigation regimes. *Plant Soil Environ.* **2015**, *61*, 6–10. [CrossRef]
50. Alam, P.; Kaur Kohli, S.; Al Balawi, T.; Altalayan, F.H.; Alam, P.; Ashraf, M.; Ahmad, P. Foliar application of 24-Epibrassinolide improves growth, ascorbate-glutathione cycle, and glyoxalase system in brown mustard (*Brassica juncea* (L.) Czern.) under cadmium toxicity. *Plants* **2020**, *9*, 1487. [CrossRef]