

Original Research

# Impact of Nitrogen, Sulphur, and Foliar Applied Thiourea on Growth, Oil Yield, and Fatty Acid Profile of Canola

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## Abstract

Canola (*Brassica napus* L) is cultivated on different types of soils, and as a result of that, many issues occur related to nutrition. Field and pot experiments were accomplished to determine canola performance in response to different sulfur and nitrogen sources. In this experiment, canola cv. "Super canola" was grown with five different nutrient treatments maintained as recommended NPK, nitrogen 220 kg ha<sup>-1</sup>, sulfur 60 kg ha<sup>-1</sup>, nitrogen-sulfur at 220-60 kg ha<sup>-1</sup> and foliar-applied thiourea at 1000 mg L<sup>-1</sup> at flowering initiation and pod formation. Application of thiourea improved the growth which was significantly similar to soil-applied N-S. Thiourea application also increased oil contents followed by a combined application of soil-applied N-S. Thiourea and N-S increased protein contents ranging from 19.28% to 23.50%. Among fatty acids, thiourea showed higher concentrations in linoleic (18.73%), oleic (58.39%), and linolenic acid (8.29%) while lowering the erucic acid concentration (2.92%).

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The highest erucic acid contents were observed when recommended NPK was applied. In conclusion, foliar-applied thiourea has the potential to boost canola growth, yield, oil quality, protein contents, and fatty acid profile while also being an economically viable and alternative source of N and S.

**Keywords:** Canola, Nitrogen and Sulphur, Leaf Area Duration, Chlorophyll, Fatty Acids, Oil Contents, Protein Content

## Introduction

Mustard and rapeseed account for most of the essential species of the genus Brassica which nearly contributes about 10% to the oilseeds and 14-15% of the consumable vegetable oil of the world. Canola is the most important in the Brassica genus, and its output has exploded due to the presence of high oil (40-45%) and protein contents (20%) [1]. Canola oil is also the most nutritious edible oil available that contains low glucosinolate contents of  $<30 \mu\text{mol g}^{-1}$  [2] and erucic acid of ~2%, and even less than 1% in some varieties, making it the best edible culinary oil [3]. The canola crop is cultivated on different types of soils, which has resulted in several nutritional problems for growers [4]. Among various stresses, nutrient stress is a limiting factor that affects plant growth and output with mineral nutrient shortages, which hampers the growth of around 60% of cultivated soils [5]. Regarding nutrient requirements, canola has very high nitrogen and sulfur requirements [6]. The importance of balanced levels of nitrogen and sulfur nutrition in canola crops has depicted a significant influence on canola yield [7]. As compared to other crops, canola requires more nitrogen because seeds and tissues of the plant body contain nitrogen in larger amounts [8]. High nitrogen rates are required and economically beneficial when canola has a suitable growth rate in irrigated fields. As a result, nitrogen fertilizers are advantageous to plants at every stage of their development [9]. Insufficient nitrogen application rates are a common cause of low canola yields [10, 11]. Nitrogen is also a component of chlorophyll, proteins, and other enzymes required for faster leaf area development, longer leaf life and duration, and overall better crop uptake and contributes to higher seed yields [12, 13]. The application of nitrogen fertilizer shows positive results by affecting key yield variables such as the number of seedpods, branches per plant, and seed weight. With respect to the development and productivity of canola crops, nitrogen presence is considered a limiting factor since nitrogen is susceptible to soil loss [14]. Sulfur is considered the second crucial nutrient after nitrogen relative to excellent growth and development of canola crops. If crops are planted in areas where Sulfur is in lower amounts, then seed quality and yield go down at a greater rate, since Sulfur is considered immobile in plants [15]. Sulfur in higher amounts affects the production of canola crops, but many crops are also affected because it is present in inadequate amounts rather than the required amount.

In recent years, the deficiency of S has become a more widespread problem due to the combination of increased cropping intensity, reduced summer fallow periods, and higher crop removal of S due to the cultivation of high-yielding cultivars, all of which require more S to achieve the high yield potential [16, 17]. Sulfur has a vital role with respect to structural and enzymatic components of plants, as protein formation occurs with the help of amino acids through the production of chlorophyll [18]. Sulfur is also required to produce a high yield and oil contents. Canola plants with a sulfur shortage grow pale yellow and develop slowly. Sulfur is directly involved with the seed yield of canola crops as 100 pounds of seed productivity occurs by only one pound of sulfur [19]. A sulfur deficit can affect crop development, seed production (up to 50% loss), and quality, and the greater yield potential of hybrid canola which increases sulfur requirements [20, 21]. Sulfur availability affects both the total concentration of glucosinolates and its different types in canola. The nitrogen supply should constantly be assessed because of the links between sulfur and nitrogen nutritional status and glucosinolate synthesis [22]. Lack of sulfur inhibits nitrogen uptake and metabolism are inextricably related to N nutrition, and plant sulfur status has a major impact on N metabolism [23, 24]. In canola, sulfur supply increased nitrogen efficiency, leading to its better N assimilation for leaf proteins [25]. For optimal production, a sufficient supply of N and S is required, as both contribute to higher seed yield quality and productivity [7]. Likewise, sulphydryl compounds such as thiourea are also used for improving assimilate partitioning, growth and development, and photosynthetic efficiency of crops [26]. The SH group in thiourea confers a regulatory characteristic, which upregulates canola crop productivity [26]. Thiourea is a nitrogen-sulfur-based fertilizer that also functions as a growth regulator in plants. During the growing stage, the application of thiourea improves plant metabolic activities like plant water relations, antioxidant redox potential, and photosynthetic rate [27]. It also aids ROS detoxification, as indicated by increased photosynthetic rate, higher yield, and enhanced source-sink connections [28]. Treatments with foliar thiourea in canola throughout critical crop phases, such as flowering and pod filling, improved shoot dry matter and photosynthetic efficiency [29].

Numerous reports demonstrate the positive impact of thiourea on crop yields in various plants. However, there is a lack of information regarding the interactive impacts of N and thiourea on canola growth, crop productivity, and oil quality. Hence, the current study aims to assess

the potential of thiourea as an alternative to sulfur combined with nitrogen focusing on its influence on the growth, yield and oil quality of two different canola cultivars.

## Materials and Methods

### Experimental Site and Crop Husbandry

Canola seeds of the cultivar Super canola were obtained from the Research Institute of Oilseed, AARI (Ayub Agriculture Research Institute), Faisalabad-Pakistan. The early seed germination percentage and moisture contents were 90% and 10.2%, respectively. The seedbed preparation was carried out by cultivating the soil 3 times using a cultivator. The soil was sandy clay loam characterized by a pH of 7.8, total nitrogen 0.045%, total exchangeable salts 1.60 dS m<sup>-1</sup>, organic matter 0.82%, available phosphorus 4.37 mg kg<sup>-1</sup> and potassium (142 mg kg<sup>-1</sup>). After the analysis of soil, nitrogen, phosphorus, and potassium were applied at 220, 110, 110 kg ha<sup>-1</sup> by using urea (applied only in treatments where nitrogen is found), TSP and muriate of potash (KCl) as a source of fertilizer, respectively. The whole application of P and K was done at the time of sowing. However, N was applied in two splits, i.e., ½ part at the time of sowing and the remaining part later at the 5-6 leaf stage keeping all other practices uniform.

### Pot Experiment

A pot experiment was done at the University of Agriculture, Faisalabad (UAF) (longitudinally 73° East, latitudinally 31° North and altitudinally at 135 m over the sea level) and the experiment was conducted by following a completely randomized design (CRD) with four replications. Pots were filled with soil collected from the representative field. About 0-15 cm upper part of the soil was collected of the soil. Collected soil was ground, air-dried and also passed through a 2 mm sieve. Each polythene-lined pot was then filled with 8 kg of soil. Ten seeds were sown per pot and four plants remained after thinning in each pot. Thiram/Captan @ 2 g kg<sup>-1</sup> fungicide was used to treat seed before sowing. Pots were irrigated to field capacity during the experiment.

### Field Experiment

The experiment was conducted at UAF (longitudinally 73° East, latitudinally 31° North and altitudinally at 135 m over the sea level) and the experiment is a randomized complete block design by using four repetitions. Each experimental plot consisted of an area of 5 m x 3 m. The average temperature during the growing and developing period ranged from 12.7°C to 25.9°C monthly and relative humidity occurred between 50.5% to 79.3%. Sowing of the crop

was done on 18 October 2020 by a hand drill in 45 cm spaced rows from each other with 5 kg ha<sup>-1</sup> seed rate. At the 3-5 leaf stage (BBCH-Scale 13-15), a spacing of 15 cm is maintained with the help of the thinning process. Sulfur was applied directly to the soil or foliage spray by using chemicals such as elemental Sulfur (60 kg ha<sup>-1</sup>) and thiourea (1,000 mg L<sup>-1</sup>), respectively. Elemental sulfur to soil at 60 kg ha<sup>-1</sup>; and thiourea to foliage at 1,000 mg L<sup>-1</sup>. Both of these treatments were applied at flowering and rosette stages. Thus, five treatments were evaluated i.e., recommended NPK 220 kg ha<sup>-1</sup>, 110 kg ha<sup>-1</sup>, 110 kg ha<sup>-1</sup> during land preparation potash and phosphorous were applied and nitrogen was divided into two splits, Nitrogen soil applied 220 kg ha<sup>-1</sup> given to the crop in two splits, 1<sup>st</sup> on sowing and 2<sup>nd</sup> dose at first water, Sulfur soil (60 kg ha<sup>-1</sup>) was applied at sowing time. Sulfur and Nitrogen were applied @ 60 kg and 220 kg respectively. Half of the nitrogen was applied at sowing with nitrogen remaining split at first irrigation, and thiourea foliarly applied about 1000 mgL<sup>-1</sup> at the time of the flowering stage. Fungicides Thiram/Captan @ 2 g kg<sup>-1</sup> seed was applied to treat the seeds just before sowing. Three irrigations were applied to the crop i.e. 1<sup>st</sup> irrigation 30 DAS and then each with 30 days interval.

### Crop Rowth and Physiological Attribute

The parameters related to growth and development, area unit of 1 m<sup>2</sup> from the field and two plants randomly from pots were harvested 30 DAS frequently with each 15-day gap. The plants were divided into two parts i.e. stem and root and placed in a hot air oven (WFO-600, Tokyo Rikakikai Co. Ltd) at 70°C until no moisture content was left. Leaf area meter (AM-350, CID, Inc. USA) was used as described by [30] to measure the leaf area. The methodology of [31] was used to calculate the leaf area duration (LAD). Both were used to predict the crop growth rate (CGR) and net assimilation rate (NAR) according to [31]. For the analysis of chlorophyll in leaf, samples of plants were taken 30 DAS with 15 days intervals and determined the following [32] method. The Whatman No. 42 filter paper was used for filtration to conduct this parameter. Approximately 1 g of fresh plant material was taken, and its extract was added to a 10 mL solution of 80% acetone. The spectrophotometer (Campspec M501 Single Beam UV/vis Spectrophotometer, UK) was used to measure the absorbance (A) rates at 485, 645, and 663 nm chlorophyll a, chlorophyll b, and carotenoids, respectively. Chlorophyll and carotenoid levels were determined using the following equation:

$$\text{Chl } a = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Chl } b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Carotenoids} = [A_{485} + 0.114 (A_{663}) - 0.638(A_{645})]$$

## Agronomic and Yield-Related Traits

Morphological parameters such as plant height, branches and pods plant<sup>-1</sup> were measured at the time of maturity. Yield and related traits were determined using the standard procedures. The crop was harvested and threshed when light brown color appeared in siliqua. For the separation of seeds winnowed was used. Harvest index, seed per siliqua, grain yield, and 1000-seed weight were estimated.

## Seed Oil Quality Attributes

Soxhlet method [33] was used to determine oil content. Kjeldahl method [34], was followed for the determination of proteins in seeds. Crude protein contents were estimated by acid-based titration and volume of acid. Fatty acid profile was used to determine by following the worldwide oil standard ISO 5509:2000. Gas chromatograph (Varian 5890) with a capillary column, CP-Sil 88 is used and fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

## Statistical Analysis

Statistical analyses were applied to the collected data using the software Statistix 10.1. The significance of variance was determined by using ANOVA and Treatment means were compared by LSD test (at %5 probability). Graphs were drawn using Sigma plot (Systat Software, San Jose, CA.). Standard deviations were calculated using MS-Excel software.

## Results

### Growth and Physiological Parameter

Both Nitrogen and Sulfur applications had significant impacts on the growth and yield of canola crops. All growth and yield attributes were considerably affected under different sulfur and nitrogen sources as compared to control but thiourea performed better as compared to soil applications. Maximum CGR was observed with foliar application of thiourea (1,000 mg L<sup>-1</sup>) and directly applied in the soil. Nitrogen-sulfur at all harvests is associated with increased crop growth in the current study (Fig. 1A). Application of thiourea and soil applied nitrogen and sulfur significantly increased NAR. Soil applied Sulfur-Nitrogen and foliar applied thiourea (1,000 mg L<sup>-1</sup>) also produced maximum LAI, respectively as indicated by NAR and CGR (Fig. 1 A, B, C). Maximum LAD was determined from soil-applied Sulfur-Nitrogen which was comparable to foliar-applied thiourea in super canola (Fig. 3 A). However, without any other nutrient applied minimum NAR, LAD, CGR, and LAI were recorded (Figs. 1,

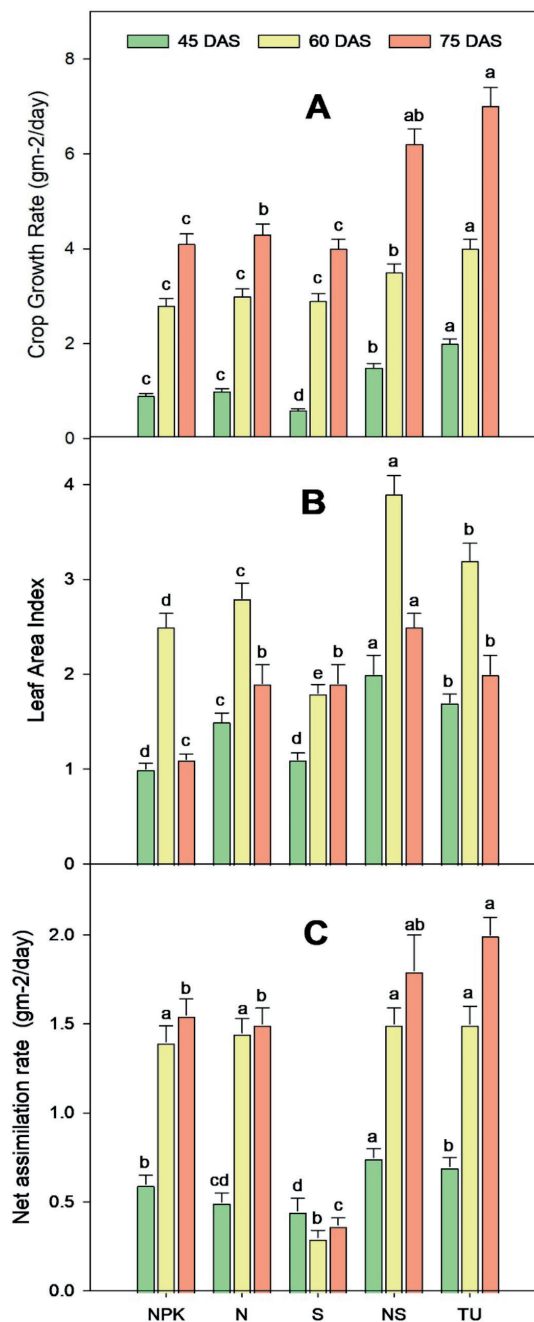


Fig. 1. Crop growth rate ( $\text{g m}^{-2} \text{day}^{-1}$ ) (A), Leaf area index (B) and Net assimilation rate ( $\text{g m}^{-2} \text{day}^{-1}$ ) (C) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means  $\pm$  SD.

A, B, C and Fig. 3 A). Maximum Chlorophyll a and b contents were found from thiourea at each crop harvest following soil applied N and S with maximum contents at 75 DAS. Carotenoids similar to chlorophyll were also found to increase in foliar applied thiourea (1000 mg L<sup>-1</sup>) at each crop harvest followed by soil-applied Nitrogen and Sulfur, especially at 75 DAS. However, minimum Chl (a, b) and carotenoid contents were produced by soil alone S at each harvest with no other nutrient applied (Fig. 2 A, B, C).

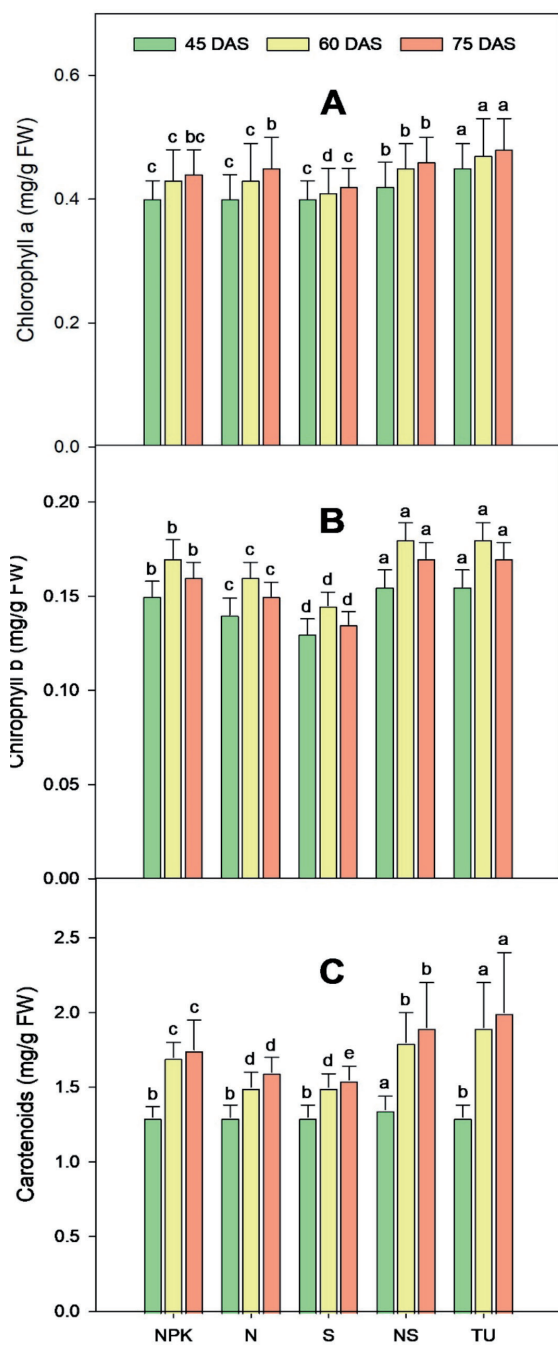


Fig. 2. Chlorophyll a ( $\text{mg g}^{-1}$  f wt.) (A), Chlorophyll b ( $\text{mg g}^{-1}$  f wt.) (B), and Carotenoids ( $\text{mg g}^{-1}$  f wt.) (C) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means $\pm$ SD.

### Agronomic and Yield Traits

Sulfur and nitrogen application increased plant height. Maximum plant height was predicted relative to foliar application of thiourea ( $1,000 \text{ mg L}^{-1}$ ) comparable to soil-applied Nitrogen and Sulfur (Table 1). Likewise, the number of branches was similar for foliage-applied thiourea ( $1,000 \text{ mg L}^{-1}$ ) and soil-applied N and S. Nonetheless, the crop attained a greater number of pods with foliar-applied thiourea ( $1,000 \text{ mg L}^{-1}$ ) at maturity

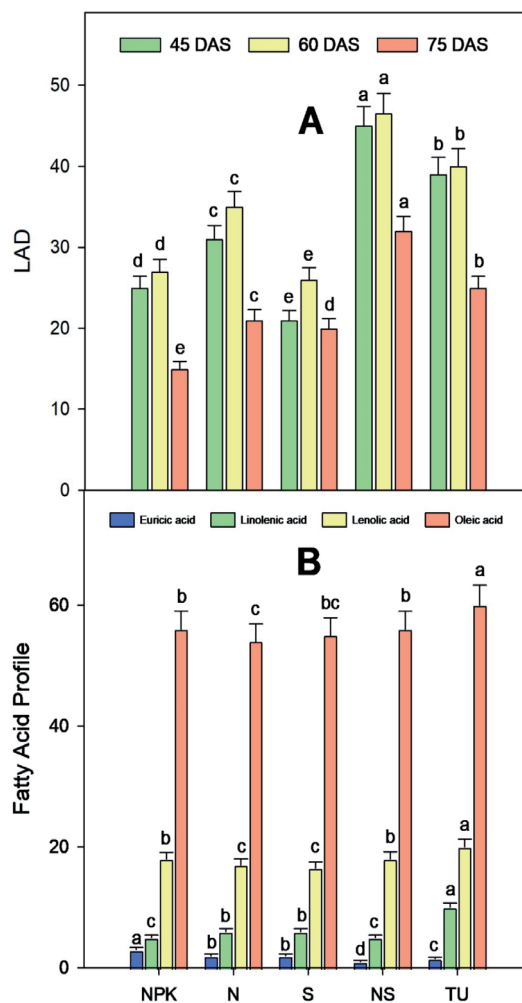


Fig. 3. Leaf area duration (days) (A), and Fatty acid profile (B) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means $\pm$ SD.

time (Table 1). Likewise, thiourea and nitrogen and sulfur application increased yield and yield-related attributes as indicated by significant interaction between treatments (Table 1). Maximum seeds per silique were also found in the case of foliage applied thiourea ( $1,000 \text{ mg L}^{-1}$ ) at maturity time which resulted in an increased 1,000-seed weight. Application of thiourea ( $1,000 \text{ mg L}^{-1}$ ) as well as soil application of Nitrogen and Sulfur maximum increased seed yield and harvest index in canola (Table 1). These positive results in yield attributes were obtained by the application of NPK and minimum for soil-applied Sulfur. However, significantly higher results were obtained in yield-related attributes traits for foliar-applied thiourea ( $1,000 \text{ mg L}^{-1}$ ) (Table 1).

### Seed Oil Quality

Seed oil and protein contents increased by the application of Sulfur through soil or foliar thiourea. Foliar-applied thiourea ( $1,000 \text{ mg L}^{-1}$ ) followed by soil-applied Nitrogen and Sulfur in super canola showed

Table 1. Influence of different soil-applied N-S and foliar Thiourea application on growth and yield parameters of super canola in the field Experiment.

| Treatment            | PH (cm) | NBP     | NPP    | NSP    | 1000-GW (g) | SY (t/ha) | BY (t/ha) | HI       |
|----------------------|---------|---------|--------|--------|-------------|-----------|-----------|----------|
| NPK (220:110:110)    | 156.8bc | 11.5bc  | 390abc | 25.0ab | 4.9abc      | 2.2abc    | 8.86bc    | 24.9 abc |
| N (220 Kg/ha)        | 160.7bc | 12.3abc | 375bc  | 21.7bc | 4.8bc       | 2.1bc     | 9.25abc   | 22.4bc   |
| S (60 kg/ha)         | 144.3c  | 10.0c   | 344c   | 20.5c  | 4.6c        | 1.7c      | 8.20c     | 20.5c    |
| N-S (220-60 kg/ha)   | 170.2ab | 13.5ab  | 408ab  | 22.7bc | 5.5ab       | 2.6ab     | 9.80ab    | 27.0ab   |
| Thiourea (1000 mg/L) | 185.2a  | 14.7a   | 436a   | 27.5a  | 5.6a        | 2.8a      | 9.82a     | 28.8a    |

Table 2. Influence of different soil-applied N-S and foliar Thiourea application on oil quality parameters and fatty acid profile of Super Canola in the field experiment.

| Treatment            | Oil Content (%) | Protein Content (%) | Oleic acid | Linolic acid | Linolenic acid | Euricic acid |
|----------------------|-----------------|---------------------|------------|--------------|----------------|--------------|
| NPK (220:110:110)    | 39.390 bc       | 19.280 bc           | 56.32 ab   | 17.80 ab     | 5.82 c         | 2.920 a      |
| N (220 Kg/ha)        | 35.010 c        | 18.808 bc           | 53.19 c    | 16.723 bc    | 6.95 bc        | 1.75 bc      |
| S (60 kg/ha)         | 41.570 bc       | 17.180 c            | 54.63 bc   | 15.780 c     | 7.580 ab       | 2.210 b      |
| N-S (220-60 kg/ha)   | 42.095 ab       | 21.680 ab           | 55.96 ab   | 17.290 b     | 7.710 ab       | 1.38 c       |
| Thiourea (1000 mg/L) | 46.567 a        | 23.350 a            | 58.39 a    | 18.735 a     | 8.290 a        | 1.92 bc      |

the highest seed oil and protein contents. Minimum oil contents were found for nitrogen nutrition with no other nutrient added while the minimum amount of protein was found in nutrition of Sulfur (Table 2). Nitrogen and Sulfur nutrition also improved the oil fatty acid profile and maximum contents were predicted for foliar thiourea and soil direct application of nitrogen and sulfur in combination. However, the maximum increase in oil oleic acid but the lowest levels of euricic acid were found for foliar-applied thiourea (1000 mg L<sup>-1</sup>). Linolic and linoleic acids were increased for foliar application of thiourea (1000 mg L<sup>-1</sup>) while minimum oil oleic acid, linoleic acid, and linolic and euricic acid were found in alone nutrition of nitrogen (Fig. 3 B).

## Pot Experiment

### Crop Growth and Chlorophyll Contents

Application of Nitrogen and Sulfur, also significant growth of canola in the case of pots but to a lesser extent as compared to the field results. Para-mounted CGR was recorded in pots for foliar application of thiourea (1,000 mg L<sup>-1</sup>) while soil direct application of Nitrogen and Sulfur at all harvests was associated with the increased crop growth (Figs. 4 A). On par with CGR application of 1,000 mg L<sup>-1</sup> of thiourea while soil-applied nitrogen and sulfur significantly increased NAR also in the pots. Direct application in soil (Sulfur and Nitrogen) and foliar application of thiourea (1,000 mg L<sup>-1</sup>)

produced all-out LAI, respectively as indicated by NAR and CGR similar to field (Fig. 4 A, B, C). LAD was recorded increased from soil applied Sulfur and Nitrogen while similar to foliar application of thiourea (1,000 mg L<sup>-1</sup>) in super canola as associated with the leaf area index and photosynthesis also indicated in pots (Fig. 6). However, minimum CGR, LAI, NAR and LAD were recorded for Sulfur alone without any other application of nutrient (Figs. 4, A, B, C and Fig. 6). Increased content of Chlorophyll a and chlorophyll b were estimated from 1,000 mg L<sup>-1</sup> of thiourea at each harvest of each crop also showed similar to field in pots following for soil applied N and S with maximum contents at 75 DAS as it is due to the higher efficacy of nitrogen and sulfur associated in the formation of chlorophyll structure and pigments. Carotenoids similar to chlorophyll were also found to increase in 1,000 mg L<sup>-1</sup> thiourea at each crop harvest in pots following direct soil application of Nitrogen and Sulfur with maximum contents at 75 DAS. However, the lowest Chlorophyll a, chlorophyll b and carotenoid contents were produced by soil alone S at each harvest in pots with no other nutrient applied linked to the lack of other nutrients at 75 DAS (Fig. 5 A, B, C).

### Agronomic and Yield Traits

Agronomic traits overall increased due to the application of Sulfur and Nitrogen. Both applications of Sulfur and Nitrogen increased plant height. Maximum plant height was estimated for foliar application of

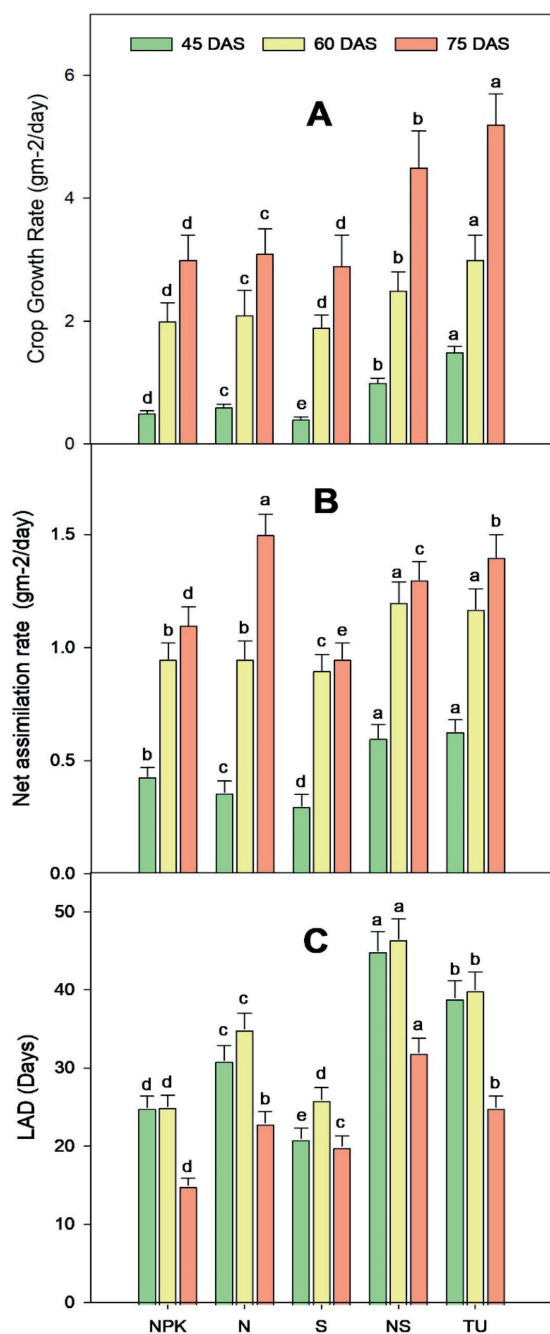


Fig. 4. Crop growth rate ( $\text{g m}^{-2} \text{day}^{-1}$ ) (A), Leaf area index (B), and Net assimilation rate ( $\text{g m}^{-2} \text{day}^{-1}$ ) (C) of Super Canola under different combinations of N-S and Thiourea in the pots experiment. Values represent means $\pm$ SD.

thiourea direct soil application of Nitrogen and Sulfur (Table 1). Similarly, foliar application of  $1,000 \text{ mg L}^{-1}$  of thiourea and direct soil application of Nitrogen and Sulfur produced a similar number of branches per plant. Nonetheless, the number of pods per plant attained in greater numbers in case of foliar application of  $1,000 \text{ mg L}^{-1}$  thiourea in pots at the time of maturity (Table 3). Therefore, agronomic and yield traits increased due to thiourea foliage and Nitrogen-Sulfur application through the soil in pots as predicted by

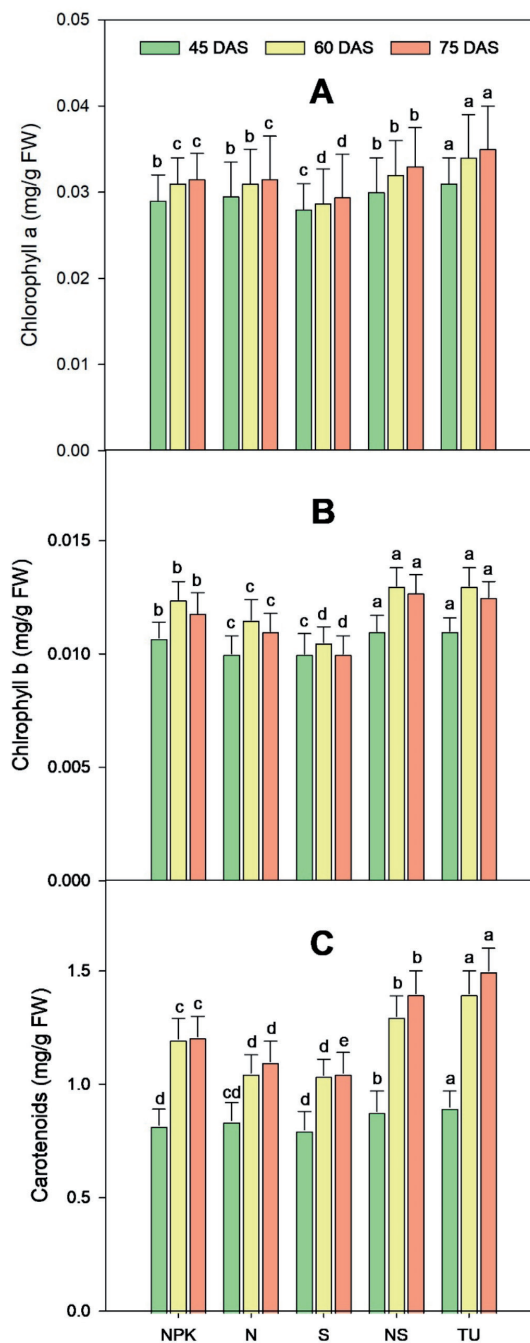


Fig. 5. Chlorophyll a ( $\text{mg g}^{-1} \text{f wt.}$ ) (A), Chlorophyll b ( $\text{mg g}^{-1} \text{f wt.}$ ) (B), and Carotenoids ( $\text{mg g}^{-1} \text{f wt.}$ ) (C) of Super Canola under different combinations of N-S and Thiourea in the pot experiment. Values represent means $\pm$ SD

a significant interaction between treatments (Table 3). The number of seeds per silique was also found to increase in the case of foliar application of  $1,000 \text{ mg L}^{-1}$  thiourea at maturity time which resulted in increased seed size and also increased 1,000-seed weight, seed yield, and harvest index. It was observed that positive results by the application of  $1,000 \text{ mg L}^{-1}$  thiourea with soil application of Sulphur and Nitrogen in canola plants were linked with all increased yield parameters among pots (Table 3). It was evident from ANOVA

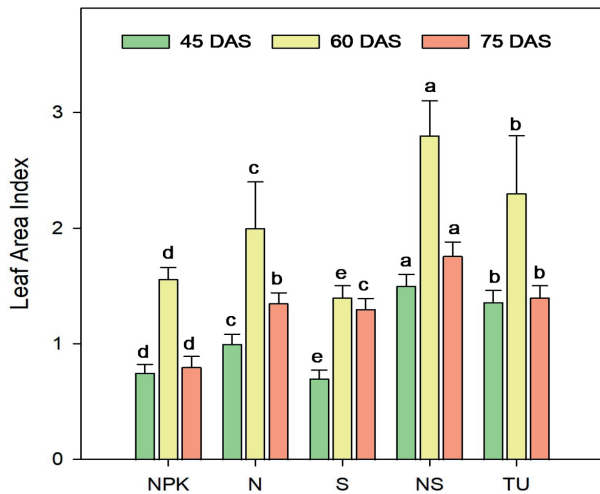


Fig. 6. Leaf area duration (days) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means $\pm$ SD.

that significant results were obtained in agronomic and yield-related traits with foliar application of 1000 mg L<sup>-1</sup> thiourea (Table 3). Foliar applied thiourea improved the benefits of production as compared to the soil applied as much of the losses take place during nitrogenous fertilizer application and soil micro-flora activities. Higher benefits were obtained by the application of 1000 mg L<sup>-1</sup> thiourea followed soil soil-applied nitrogen-sulfur. The seed oil quality was not measured because of the small number of seeds for the analysis process.

## Discussion

Sulfur and Nitrogen application by thiourea or soil-applied fertilizers enhanced the crop yield and improved seed quality in canola. According to the present study, Super Canola shows remarkable results i.e. increased LAI with soil application of Sulfur and Nitrogen. It is assumed that foliar-applied thiourea enhanced the cell division, expansion of leaves and light interception which consequently improved the vegetative growth of crop plants [35, 36]. Increased leaf area results in

better growth, light interception and larger leaf area duration with thiourea regulating the physiological and antioxidant defense mechanisms relative to gene expression [37]. The application of thiourea led to an increase in LAD. This could be attributed to the role of S application in leaf senescence, which possibly delayed the senescence process, as evidenced by delayed flowering and maturity [37]. The application of Thiourea resulted in an increase in leaf area, facilitating greater production and distribution of photo-assimilates to various sinks, ultimately leading to a higher crop growth rate and net assimilation rate [26]. Crop growth rate and net assimilation rate are impacted by the application of thiourea, which enhances the regulation of source-to-sink connection. This, in turn, promotes the translocation of metabolites from the leaves (source) to the pods (sink), resulting in increased efficiency [38] (Fig. 2). In the present study thiourea application clearly enhanced the chlorophyll contents as thiourea might be involved in the regulation of plant metabolic activities [39]. Likewise in sunflower cultivars, thiourea enhanced growth and photosynthetic pigments that improved the photosystem activity along with the expression of antioxidants in the enzymatic and non-enzymatic systems [40]. Previous observation showed that thiourea enhanced the chlorophyll content of leaves up to 4% as compared to control in maize. The foliar application of a 1000 ppm thiourea solution on maize crops resulted in a significant increase in chlorophyll content, as compared to plants that were sprayed with water [41]. Foliar spray of thiourea at 75 and 90 DAS, clear increase in growth, yield and photosynthetic pigment in canola in the present study might be due to the increase in chlorophyll [42, 43]. The higher LAD after thiourea foliar application was due to higher chlorophyll contents, reducing sugars, starch, and soluble proteins, as well as delaying leaf aging and senescence [44]. Increased shoot length in the current study might be due to the application of thiourea on leaves because it has stimulatory effects on cell division [45]. Increased plant height by sulfur nutrition about 40 kg ha<sup>-1</sup> in canola was already reported in a previous study [46]. A similar study also concluded increased plant length by the application of 1,000 mg L<sup>-1</sup> thiourea in cowpeas. More branches and pods are reported due to the application of

Table 3. Influence of different soil-applied N-S and foliar thiourea application on growth and yield parameters of Super Canola in the pot experiment.

| Treatment           | PH (cm) | NBP    | NPP       | NSP    | 1000-GW (g) | SY (t/ha) | BY (t/ha) | HI     |
|---------------------|---------|--------|-----------|--------|-------------|-----------|-----------|--------|
| NPK (220:110:110)   | 95.7bc  | 6.2bc  | 145.00 bc | 19.0b  | 4.21bc      | 0.82bc    | 3.29bc    | 24.0bc |
| N (220 Kg/ha)       | 101.3b  | 7.3bc  | 132.00 cd | 17.3bc | 4.01cd      | 0.72cd    | 3.26cd    | 22.1c  |
| S (60 kg/ha)        | 85.3c   | 4.7c   | 118.00 d  | 13.0c  | 3.4d        | 0.48d     | 2.80d     | 17.3d  |
| N-S (220-60 kg/ha)  | 107.0ab | 8.7 ab | 161.34 ab | 21.3ab | 4.6ab       | 1.04ab    | 3.87ab    | 27.0ab |
| Thiourea (1000mg/L) | 120.0 a | 11.0a  | 173.33 a  | 24.0a  | 4.9a        | 1.07a     | 3.90a     | 27.6a  |



thiourea on leaves because of the increased source-to-sink ratio due to sulfur nutrition and overall improved characteristics of the photosynthetic machinery. The current study showed that thiourea at the rate of 1000 mg L<sup>-1</sup> showed significantly higher leaves per plant, plant height, fertile tillers, spikelets per spike, and yield-associated attributes in both pot and field experiments. It might be a result of improved partitioning of total DM into grain. As sulfur plays a crucial role in the assimilates production has already been reported in soybean [47]. Similarly, increased seed yield and straw yield in canola were due to the foliar spray of thiourea. At the pre-flowering and pod initiation stages, the foliar application of thiourea (1000 ppm) resulted in significant improvements in vegetative growth and yield-attributing characteristics, leading to increased yields of both grain and straw. Furthermore, thiourea treatment improved protein content and plant nutrient (N, P, K, and S) uptake [48]. The application of thiourea increased the number of seeds in canola due to aid in the effective transfer of photoassimilates from source to sink. According to [49], thiourea is engaged in protein synthesis and net assimilates production, as well as aiding in the consumption of N and P during seed formation. [46] also found that applying thiourea spray markedly enhanced the number of seeds in canola. Thiourea application increased protein synthesis and oil content creation due to sulfur involvement in the production of proteins and oil contents, as well as assisting in the consumption of N and P during seed formation, according to [50], which resulted in a remarkable increase 1000-grain weight. Foliar-applied thiourea enhanced yield by modifying physiological systems. Foliar applied thiourea at the pre-flowering stage in pearl millet, improved crop growth and DM yield dramatically [51]. [9] reported a significant increase in biological yield and harvest index when thiourea was applied to the wheat crop. Another research conducted by [46] suggested that foliar applied sulphahydral compounds (like thiourea) clearly enhanced yield and harvest index in canola. Thiourea increased oil contents and fatty acid profile because sulfur is a component of oil molecules, and it boosts the oil content of most oilseeds as sulfur fertilization an average increased 5% in oil content and fatty acid profile [52]. Fatty acid profile increased as sulfur is also involved in the synthesis of glucosides or glucosinolates in oil seed crops which when hydrolyzed increase the oil content [53]. The usage of thiourea increases the number of glucosinolates in the seed, which boosts the volume and quality of oil produced in canola [46, 54]. [55], observed that the impacts of Nitrogen and Sulfur on protein content in canola were considerable, whereas the influence of delivery methods was not significant. The increased fatty acid profile might be due to frequent supply of S through foliar application of thiourea.

## Conclusions

In conclusion, thiourea (1000 mgL<sup>-1</sup>) foliar application increased growth yield and agronomic attributes at a distinctive level with increasing quality parameters of oil and protein contents along with the fatty acid profile. Thiourea proved to be a potential alternative to increase the qualitative and quantitative attributes of canola crops to enhance canola productivity and performance around the globe.

## List of Abbreviations

|            |                                 |
|------------|---------------------------------|
| NPK        | Nitrogen, Phosphorus, Potassium |
| N-S        | Nitrogen-Sulfur                 |
| SH         | Thiol Groups                    |
| TSP        | Triple Superphosphate           |
| DAS        | Days After Sowing               |
| FAME       | Fatty Acid Methyl Esters        |
| CGR        | Crop Growth Rate                |
| NAR        | Net Assimilation Rate           |
| LAI        | Leaf Area Index                 |
| N          | Nitrogen                        |
| S          | Sulfur                          |
| <i>Chl</i> | Chlorophyll                     |
| LAD        | Leaf Area Duration              |
| CRD        | Completely Randomized Design    |
| DM         | Dry Matter                      |

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## Conflict of Interest

The authors declare no conflict of interest.

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