ONLINE PUBLICATION DATE:

Original Research

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

Muhammad Usama Rasheed¹, Wang Lihong^{2*}, Athar Mahmood ^{1**}, Hafeez Ur Rehman¹, Muhammad Mansoor Javaid³, Basharat Ali⁴, Filza Ishfaq⁵, Muaz Ameen⁵, Muhammad Umair Hassan⁶, Abeer Hashem⁷, Khalid F. Almutairi⁸, Elsayed Fathi Abd_Allah⁸

 ¹Department of Agronomy, University of Agriculture Faisalabad, 38000, Faisalabad, Pakistan
²College of Tourism and Geographic Sciences, Baicheng Normal University, Baicheng 13700, Jilin, China
³Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha, Pakistan
⁴Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 62400, Pakistan
⁵Department of Botany, University of Agriculture Faisalabad, 38000, Faisalabad, Pakistan
⁶Research Center on Ecological Sciences, Jiangxi Agricultural University, Nanchang China
⁷Botany and Microbiology Department, College of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia
⁸Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia

> Received: 25 November 2023 Accepted: 22 March 2024

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⁻¹, sulfur 60 kg ha⁻¹, nitrogen-sulfur at 220-60 kg ha⁻¹ and foliar-applied thiourea at 1000 mg L⁻¹ at flowering initiation and pod formation. Application of thiourea improved the growth which was significantly similar to soil-applied N-S. Thiourea and N-S increased oil contents followed by a combined application of soil-applied N-S. Thiourea showed higher concentrations in linoleic (18.73%), oleic (58.39%), and linolenic acid (8.29%) while lowering the erucic acid concentration (2.92%).

*e-mail: wlh19721108@163.com

^{**}e-mail: athar.mahmood@uaf.edu.pk

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 µmol 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 highyielding 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, sulfhydral 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 sourcesink 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⁻¹, organic matter 0.82%, available phosphorus 4.37 mg kg⁻¹ and potassium (142 mg kg⁻¹).. After the analysis of soil, nitrogen, phosphorus, and potassium were applied at 220, 110, 110 kg ha⁻¹ 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., 1/2 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 attitudinally 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⁻¹ 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 attitudinally 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⁻¹ 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⁻¹) and thiourea (1,000 mg L⁻¹), respectively. Elemental sulfur to soil at 60 kg ha⁻¹; and thiourea to foliage at 1,000 mg L⁻¹. Both of these treatments were applied at flowering and rosette stages. Thus, five treatments were evaluated i.e., recommended NPK 220 kg ha⁻¹, 110 kg ha⁻¹, 110 kg ha⁻¹ during land preparation potash and phosphorous were applied and nitrogen was divided into two splits, Nitrogen soil applied 220 kg ha⁻¹ given to the crop in two splits, 1st on sowing and 2nd dose at first water, Sulfur soil (60 kg ha⁻¹) 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⁻¹ at the time of the flowering stage. Fungicides Thiram/Captan @ 2 g kg-1 seed was applied to treat the seeds just before sowing. Three irrigations were applied to the crop i.e. 1st 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⁻² 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:

 $Chl_{a} = 12.7 \text{ x } A_{663} - 2.69 \text{ x } A_{645}$ $Chl_{b} = 22.9 \text{ x } A_{645} - 4.68 \text{ x } A_{663}$ $Carotenoids = [A_{485} + 0.114 (A_{663}) - 0.638(A_{645})]$

Agronomic and Yield-Related Traits

Morphological parameters such as plant height, branches and pods plant⁻¹ 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⁻¹) and directly applied in the soil. Nitrogensulfur 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-1) 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,

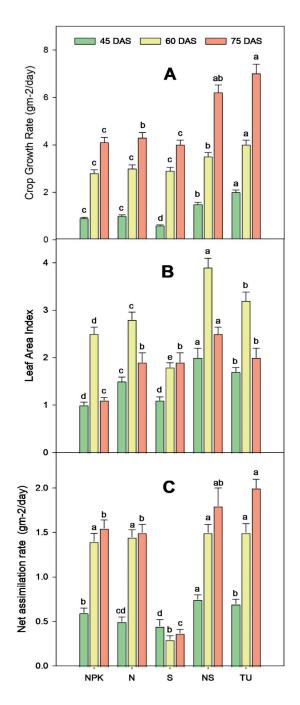


Fig. 1. Crop growth rate (g $m^{-2} day^{-1}$) (A), Leaf area index (B) and Net assimilation rate (g $m^{-2} day^{-1}$) (C) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means±SD.

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⁻¹) 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).

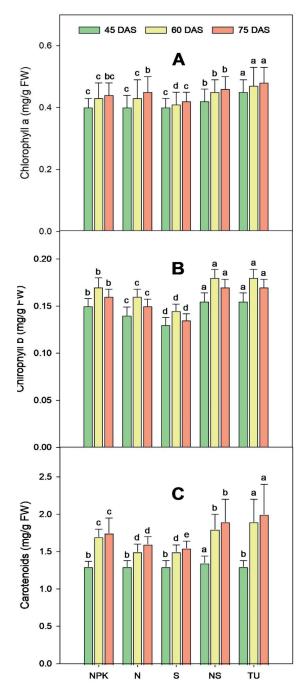


Fig. 2. Chlorophyll a (mg g⁻¹ f wt.) (A), Chlorophyll b (mg g⁻¹ f wt.) (B), and Carotenoids (mg g⁻¹ f wt.) (C) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means \pm SD.

Agronomic and Yield Traits

Sulfur and nitrogen application increased plant height. Maximum plant height was predicted relative to foliar application of thiourea (1,000 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 mg L⁻¹) and soil-applied N and S. Nonetheless, the crop attained a greater number of pods with foliar-applied thiourea (1,000 mg L⁻¹) at maturity

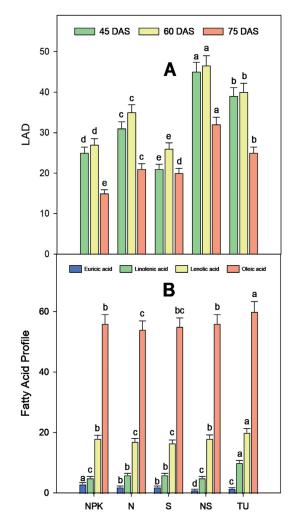


Fig. 3. Leaf area duration (days) (A), and Fatty acid profile (B) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means±SD.

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 siliqua were also found in the case of foliage applied thiourea (1,000 mg L⁻¹) at maturity time which resulted is an increased 1,000-seed weight. Application of thiourea (1,000 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 (1000 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 (1000 mg L⁻¹) followed by soil-applied Nitrogen and Sulfur in super canola showed

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

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⁻¹). Linolic and linoleic acids were increased for foliar application of thiourea (1000 mg L⁻¹) 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 $(1,000 \text{ mg L}^{-1})$ 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⁻¹ 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 $(1,000 \text{ mg L}^{-1})$ 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⁻¹) 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⁻¹ 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-1 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

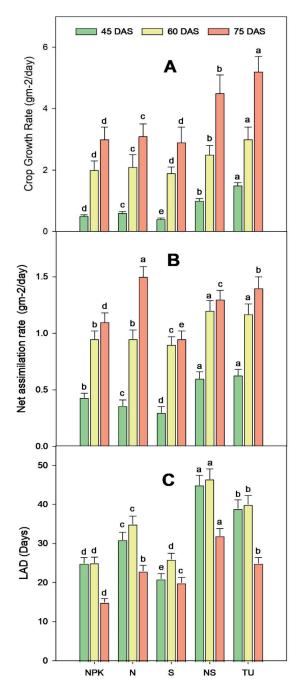


Fig. 4. Crop growth rate $(g m^{-2} day^{-1})$ (A), Leaf area index (B), and Net assimilation rate $(g m^{-2} day^{-1})$ (C) of Super Canola under different combinations of N-S and Thiourea in the pots experiment. Values represent means±SD.

thiourea direct soil application of Nitrogen and Sulfur (Table 1). Similarly, foliar application of 1,000 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 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

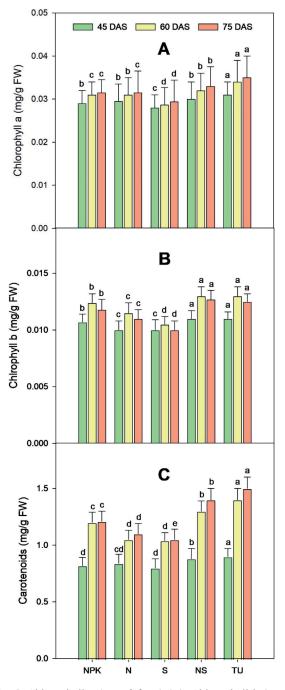


Fig. 5. Chlorophyll a (mg g^{-1} f wt.) (A), Chlorophyll b (mg g^{-1} f wt.) (B), and Carotenoids (mg g^{-1} f wt.) (C) of Super Canola under different combinations of N-S and Thiourea in the pot experiment. Values represent means±SD

a significant interaction between treatments (Table 3). The number of seeds per siliqua was also found to increase in the case of foliar application of 1,000 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 mg L^{-1} thiourea with soil application of Sulpur and Nitrogen in canola plants were linked with all increased yield parameters among pots (Table 3). It was evident from ANOVA

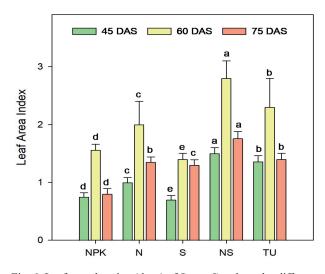


Fig. 6. Leaf area duration (days) of Super Canola under different combinations of N-S and Thiourea in the field experiment. Values represent means±SD.

that significant results were obtained in agronomic and yield-related traits with foliar application of 1000 mg L⁻¹ 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⁻¹ 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 soilapplied 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⁻¹ 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⁻¹ 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-tosink 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⁻¹ showed significantly higher leaves per plant, plant height, fertile tillers, spikelets per spike, and yieldassociated 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 sulpha-hydral 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 glucocides 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⁻¹) 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
Ν	Nitrogen
S	Sulfur
Chl	Chlorophyll
LAD	Leaf Area Duration
CRD	Completely Randomized Design
DM	Dry Matter

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

Funding

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R134), King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest

The authors declare no conflict of interest.

References

- WOODFIELD H.K., HARWOOD J.L. Oilseed Crops: Linseed, Rapeseed, Soybean, and Sunflower. Academic Press, Oxford, 2017.
- 2. ISHAQ M., RAZI R., KHAN S.A. Exploring genotypic variations for improved oil content and healthy fatty acids

composition in rapeseed (*Brassica napus* L.). Journal of Science Food and Agriculture, **97** (6), 1924, **2017**.

- 3. DORNI C., SHARMA P., SAIKIA G., LONGVAH T. Fatty acid profile of edible oils and fats consumed in India. Food Chemistry, **238**, 9, **2018**.
- ASSEFA Y., PRASAD P.V.V., FOSTER C., WRIGHT Y., YOUNG S., BRADLEY P., STAMM M., CIAMPITTI I.A. Major management factors determining spring and winter canola yield in north America. Crop Science, 58 (1), 1, 2018.
- SALEEM S., MUSHTAQ N.U., RASOOL A., SHAH W.H., TAHIR I., REHMAN R.U. Chapter two - Plant nutrition and soil fertility: physiological and molecular avenues for crop improvement. Academic Press, 2023.
- VARÉNYIOVÁ M., DUCSAY L., RYANT P. Sulphur nutrition and its effect on yield and oil content of oilseed rape (*Brassica napus* L.). Acta University Agriculture Journal, 65, (2), 2017.
- MA B.L., ZHENG Z.M., DE SILVA N., WHALEN J.K., PAGEAU D., VANASSE A., CALDWELL C., EARL H., SMITH D.L. Graphical analysis of nitrogen and sulfur supply on yield and related traits of canola in eastern Canada. Nutrienct Cycling in Agroecosystems, 118 (3), 293, 2020.
- LABRA M.H., STRUIK P.C., CALDERINI D.F., EVERS J.B. Leaf nitrogen traits in response to plant density and nitrogen supply in oilseed rape. Agronmy 10 (11), 1780, 2020.
- YADAV T., KUMAR A., YADAV R.K., YADAV G., KUMAR R., KUSHWAHA M. Salicylic acid and thiourea mitigate the salinity and drought stress on physiological traits governing yield in pearl millet- wheat. Saudi Journal of Biological Sciences, 27 (8), 2010, 2020.
- LIU Q., REN T., ZHANG Y., LI X., CONG R., WHITE P.J., LU J. Yield loss of oilseed rape (Brassica napus L.) under nitrogen deficiency is associated with underregulation of plant population density. Eurpean Journal of Agronmy, 103, 80, 2019.
- MA B.-L., BISWAS D.K., HERATH A.W., WHALEN J.K., RUAN S.Q., CALDWELL C., EARL H., VANASSE A., SCOTT P., SMITH D.L. Growth, yield, and yield components of canola as affected by nitrogen, sulfur, and boron application. Journa of Plant Nutrition and Soil Sciencee, **178** (4), 658, **2015**.
- 12. ANAS M., LIAO F., VERMA K.K., SARWAR M.A., MAHMOOD A., CHEN Z.-L., LI Q., ZENG X.-P., LIU Y., LI Y.-R. Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biology Research, 53 (1), 47, 2020.
- KAUR B., KAUR G., ASTHIR B. Biochemical aspects of nitrogen use efficiency: An overview. Journal of Plant Nutrition, 40 (4), 506, 2017.
- 14. BOUCHET A.-S., LAPERCHE A., BISSUEL-BELAYGUE C., SNOWDON R., NESI N., STAHL A. Nitrogen use efficiency in rapeseed. A review. Agronomy for Sustainable Devolopment, 36 (2), 38, 2016.
- ZENDA T., LIU S., DONG A., DUAN H. Revisiting sulphur – the once neglected nutrient: it's roles in plant growth, metabolism, stress tolerance and crop production. Agriculture, 11 (7), 626, 2021.
- SCHERER H.W. Sulphur in crop production invited paper. European Journal of Agronomy, 14 (2), 81, 2001.
- 17. SHAH F., WU W. Soil and crop management strategies to ensure higher crop productivity within sustainable environments. Sustainability, **11** (5), 1485, **2019**.

- SHAH S.H., ISLAM S., MOHAMMAD F. Sulphur as a dynamic mineral element for plants: a review. Journal of Soil Science and Plant Nutrition, 22 (2), 2118, 2022.
- PIAS O.H.D.C., TIECHER T., CHERUBIN M.R., MAZURANA M., BAYER C. Crop Yield Responses to Sulfur Fertilization in Brazilian No-Till Soils: a Systematic Review. Revista Brasileira de Ciência do Solo, 43, 2019.
- GROTH D.A., SOKÓLSKI M., JANKOWSKI K.J. A multi-criteria evaluation of the effectiveness of nitrogen and sulfur fertilization in different cultivars of winter rapeseed – productivity, economic and energy balance. Energies, 13 (18), 4654, 2020.
- RATHORE S.S., BABU S., SINGH V.K., SHEKHAWAT K., SINGH R.K., UPADHYAY P.K., HASHIM M., SHARMA K.C., JANGIR R., SINGH R. Sulfur sources mediated the growth, productivity, and nutrient acquisition ability of pearlmillet–mustard cropping systems. Sustainbility, 14 (22), 14857, 2022.
- 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. Environmental and Experimental Botany, **194**, 104739, **2022**.
- BARŁÓG P., GRZEBISZ W., ŁUKOWIAK R. Fertilizers and fertilization strategies mitigating soil factors constraining efficiency of nitrogen in plant production. Plants, 11 (14), 1855, 2022.
- 24. WU Y., LI Y., NIU L., ZHANG W., WANG L., ZHANG H. Nutrient status of integrated rice-crayfish system impacts the microbial nitrogen-transformation processes in paddy fields and rice yields. Science of Total Environment, 836, 155706, 2022.
- 25. AKMOUCHE Y., CHENEBY J., LAMBOEUF M., ELIE N., LAPERCHE A., BERTHELOOT J., D'HOOGHE P., TROUVERIE J., AVICE J.-C., ETIENNE P., BRUNEL-MUGUET S. Do nitrogen- and sulphur-remobilizationrelated parameters measured at the onset of the reproductive stage provide early indicators to adjust N and S fertilization in oilseed rape (*Brassica napus* L.) grown under N- and/or S-limiting supplies? Planta, **250** (6), 2047, **2019**.
- 26. WAQAS M.A., KAYA C., RIAZ A., FAROOQ M., NAWAZ I., WILKES A., LI Y. Potential Mechanisms of Abiotic Stress Tolerance in Crop Plants Induced by Thiourea. Frontiers in Plant Science, 10, 2019.
- 27. BISWAS D.K., MA B.L., MORRISON M.J. Changes in leaf nitrogen and phosphorus content, photosynthesis, respiration, growth, and resource use efficiency of a rapeseed cultivar as affected by drought and high temperatures. Canadian Journa of Plant Sciences, 99 (4), 488, 2019.
- 28. YANG H., LUO P. Changes in photosynthesis could provide important insight into the interaction between wheat and fungal pathogens. International Journal of Molecular Sciences, 22 (16), 8865, 2021.
- WARAICH E.A., AHMAD M., SOUFAN W., MANZOOR M.T., AHMAD Z., HABIB-UR-RAHMAN M., SABAGH A.E. Seed priming with sulfhydral thiourea enhances the performance of *Camelina sativa* L. under Heat Stress Conditions. Agronomy, **11** (9), 1875, **2021**.
- YAN G., HU R., LUO J., WEISS M., JIANG H., MU X., XIE D., ZHANG W. Review of indirect optical measurements of leaf area index: Recent advances, challenges, and perspectives. Agriculture and Forest Meterology, 265, 390, 2019.

- CHEN Y., ZHANG Z., TAO F., PALOSUO T., RÖTTER R.P. Impacts of heat stress on leaf area index and growth duration of winter wheat in the North China Plain. Field Crops Research, 222, 230, 2018.
- ANSARI F.A., AHMAD I. Fluorescent Pseudomonas -FAP2 and Bacillus licheniformis interact positively in biofilm mode enhancing plant growth and photosynthetic attributes. Scientific Reports, 9 (1), 4547, 2019.
- 33. ARAVIND S., BARIK D., RAGUPATHI P., VIGNESH G. Investigation on algae oil extraction from algae Spirogyra by Soxhlet extraction method. Materials Today: Proceedings, 43, 308, 2021.
- DROCHIOIU G., CIOBANU C.I., BANCILA S., ION L., PETRE B.A., ANDRIES C., GRADINARU R.V., MURARIU M. Ultrasound-based protein determination in maize seeds. Ultrason. Sonochem, 29, 93, 2016.
- 35. AHMAD M., WARAICH E.A., HUSSAIN S., ZULFIQAR U., TESHOME F.T., GASTELBONDO M., IMRAN M., FAROOQ M. Exogenous Application of thiourea improves the growth, seed yield, and seed fatty acid profile in late sown camelina. Journal of Soil Science and Plant Nutrition, 23 (1), 1306, 2023.
- 36. NAWAZ S., WAHID A., SHAHBAZ M., BASRA S.M.A. Evaluation of heat stress induced plant metabolites in *Fagopyrum esculentum* Moench. by exogenous application of plant growth promoters. Energy and Environment, 2, 2023.
- 37. WAHAB A., ABDI G., SALEEM M.H., ALI B., ULLAH S., SHAH W., MUMTAZ S., YASIN G., MURESAN C.C., MARC R.A. Physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: a comprehensive review. Plants, **11** (13), 1620, **2022**.
- 38. LIANG G., HUA Y., CHEN H., LUO J., XIANG H., SONG H., ZHANG Z. Increased nitrogen use efficiency via amino acid remobilization from source to sink organs in Brassica napus. The Crop Journal, **11** (1), 119, **2023**.
- 39. KAYA C., UGURLAR F., FAROOQ S., ASHRAF M., ALYEMENI M.N., AHMAD P. Combined application of asparagine and thiourea improves tolerance to lead stress in wheat by modulating AsA-GSH cycle, lead detoxification and nitrogen metabolism. Plant Physiology and Biochemistry, **190**, 119, **2022**.
- 40. MEHAK G., AKRAM N.A., ASHRAF M., KAUSHIK P., EL-SHEIKH M.A., AHMAD P. Methionine-induced regulation of growth, secondary metabolites and oxidative defense system in sunflower (*Helianthus annuus* L.) plants subjected to water deficit stress. Plos one, **16** (12), e0259585, **2021**.
- 41. SINGH T., SANDHU P.S., CHAHAL G.K., WALIA S.S. Foliar thiourea confers moisture stress tolerance in rainfed maize through elevated antioxidative defence system, osmolyte accumulation and starch synthesis grown under different planting methods. Journal of Plant Growth Regulution, 42 (1), 199, 2023.
- 42. AHMED N., RAHMAN K., RAHMAN M., SATHI K.S., ALAM M.M., NAHAR K., ISLAM M.S., HASANUZZAMAN M. Insight into the thiourea-induced drought tolerance in two chickpea varieties: Regulation of osmoprotection, reactive oxygen species metabolism and glyoxalase system. Plant Physiology and Biochemistry, 167, 449, 2021.

- 43. REHMAN H.U., ALHARBY H.F., AL-ZAHRANI H.S., BAMAGOOS A.A., ALSULAMI N.B., ALABDALLAH N.M., IQBAL T., 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, **11** (3), 371, **2022**.
- 44. FAROUK S., AL-AMRI S.M. Exogenous melatoninmediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. Ecotoxicology and Environmental Safety, 180, 333, 2019.
- 45. KAYA C., SARIOĞLU A., AKRAM N.A., ASHRAF M. Thiourea-mediated nitric oxide production enhances tolerance to boron toxicity by reducing oxidative stress in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) Plants. Journal of Plant Growth Regulation, **38** (3), 1094, **2019**.
- 46. UR REHMAN H., IQBAL Q., FAROOQ M., WAHID A., AFZAL I., BASRA S.M.A. Sulphur application improves the growth, seed yield and oil quality of canola. Acta Physiol. Plant, **35** (10), 2999, **2013**.
- 47. BAGALE S. Nutrient Management for Soybean Crops. International Journal of Agronomy, **2021**, 3304634, **2021**.
- 48. SHER A., WANG X., SATTAR A., IJAZ M., UL-ALLAH S., NASRULLAH M., BIBI Y., MANAF A., FIAZ S., QAYYUM A. Exogenous application of thiourea for improving the productivity and nutritional quality of bread wheat (*Triticum aestivum* L.). Agronomy, **11** (7), 1432, **2021**.
- WAHID A., PERVEEN M., GELANI S., BASRA S.M.A. Pretreatment of seed with H2O2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. Journal of Plant Physiology, 164 (3), 283, 2007.
- JAGETIYA B., KAUR M. Role of thiourea in improving productivity of soybean. International Journal Plant Sciences, 1 (2), 308, 2006.
- PARIHAR G., SAHU M., JOSHI N. Nitrogen, Sulphur and thiourea nutrition of pearl millet [*pennisetum glaucum* (l.) r. br.] i. effect on growth and dry matter production. Annals of Arid Zone, **36** (4), **1997**.
- 52. NAIKNAWARE M., PAWAR G., MURUMKAR S. Effect of varying levels of boron and sulphur on growth, yield and quality of summer groundnut (*Arachis hypogea* L.). Journal of Tropical Agriculture, **33** (2 (Part I)), 471, **2015**.
- IMRAN A.A.K., INAMULLAH H.Z., FAYAZ AHMAD S.T.S., AMJAD USMAN I. Yield and yield attributes of rapeseed cultivars as influence by sulfur level under Swat valley conditions. Pure and Applied Biology, 4 (3), 296, 2015.
- MANAF A., QADIR G., BASRA S. Effects of sulphur on seed yield, oil, protein and glucosinolates of canola cultivars. International Journal of Agriculture and Biology, 3, 504, 2007.
- 55. AHMAD G., JAN A., ARIF M., JAN M.T., KHATTAK R.A. Influence of nitrogen and sulfur fertilization on quality of canola (*Brassica napus* L.) under rainfed conditions. Journal of Zhejiang University of Science, 8 (10), 731, 2007.