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ADJUSTING THE ANTIOXIDANT DEFENSE SYSTEM BY SULFUR-CONTAINING COMPOUNDS TO IMPROVE THE GROWTH AND YIELD OF FLAX UNDER SANDY SOIL CONDITIONS

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SUMMARY

Cysteine and thiourea represent natural antioxidants (compounds containing sulfur) that can protect plants against a broad spectrum of environmental stresses. A field trial directly assessed the probable influence of foliar treatment of cysteine (50, 75, and 100 mg L^{-1}) and thiourea (200, 400, and 600 mg L^{-1}) on growth parameters, photosynthetic pigments, seed yield quantity and quality, and some biochemical characteristics of flax plants grown in sandy soil. A foliar spray of cysteine and thiourea markedly increased growth characters, concurrently with an increase in Indole acetic acid content and photosynthetic pigments. Compatible osmolytes and yield components also improved compared with untreated plants. All treatments increased seed yield, oil content, and its components. The ratio of unsaturated fatty acids to saturated fatty acids bore enrichment in the yielded seeds. It was also noticeable that the 400 mg L^{-1} thiourea was the most pronounced in increasing to maximum the tested parameters of the flax plant. It could be a conclusion that foliar sprays of cysteine and thiourea were active in ameliorating flax performance through increasing antioxidant compounds and enzymes.

Keywords: Flax, cysteine, thiourea, growth, osmoprotectant, antioxidants, yield, fatty acids

Key findings: The flax seed variety (Sakha-3) exhibited its maximum growth and yield by foliar spraying with 400 mg L^{-1} thiourea or 75 mg L^{-1} cysteine when cultivated in sandy soils, and this effect came as a response to improving the antioxidant defense system.

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INTRODUCTION

Global variation effects are the primary anxiety in various developing countries, with a limited capacity to improve the effects of climate change's reduction on plant production (Raza *et al*., 2019). For the urgent need to meet food and address demands in Egypt, it is imperative to cultivate additional desert regions, particularly those composed of sandy or calcareous soil. Notably, sandy soils constitute over 70% of Egypt's land area. These soil characteristics consist of deficiencies in their physical, biological, and chemical properties, having challenges in terms of water-plant interactions and nutrient content (Abou-Hadid *et al.*, 2010). One of the most traditional plants grown in Egypt is flax (*Linum usitatissimum* L.), commonly grown as a seed, fiber, and dual-purpose plant (fibers and seeds). Flax seeds contain 41% oil, 20% protein, and a high percentage of essential fatty acids (Bakry *et al.*, 2012).

Studies have shown that sulfur-containing molecules and a number of non-protein and protein thiols play an essential role in plant tolerance to different abiotic stresses (Ahmad *et al*., 2022). Cysteine, an ɑ-amino acid, consists of the thiol chain (-SH) participating in enzymatic reactions, such as nucleophile and papain-like cysteine proteases becoming involved in several plant cell procedures (Richau *et al.*, 2012). It is the precursor to glutathione (an antioxidant), as well as, the function and specific protection compounds of glucosinolates and thionins (Künstler *et al.*, 2020)**.** These compounds directly or indirectly work in the redox signaling pathway and stress tolerance mechanisms (Erdala and Turk, 2016).

Thiourea (TU), consisting of sulfur (42%) and nitrogen (36%) components, boasts improved water solubility and absorption capabilities that confer resilience against abiotic stresses. Also, these constituents increase pigment contents and photosynthesis processes (Wahid *et al.*, 2107). Additionally, the stimulatory effects of TU may be through gene expression alteration and anti-oxidative defense production, found in histological variations, osmolyte productions, water and nutritional correlations, and leaf gas substitution properties under unfavorable conditions. Ahmad *et al*. (2022) found that applying canola plants with TU significantly stimulated the nutritional value and seed quality by promoting total soluble sugars, proline contents, total soluble protein, antioxidant enzymes, and oil percent, consequently improving yield-contributing parameters. This research investigated the effectiveness of using different concentrations of cysteine and thiourea as a foliar spray on growth, some biochemical aspects, and yield quantity and quality of flax planting in sandy soil.

MATERIALS AND METHODS

Experiment location

Two field experiments commenced in two sequential winter seasons in 2019–2020 and 2020–2021 at the National Research Center's experimental farm in the Nubaria region of Egypt (30_86'67" N 31_16 ' 67 " E, with a mean altitude of 21 masl). The zone of the soil farm may be arid or semi-arid. At night, temperatures ranged from 10.12 °C to 16.54 °C, with an average of 12.81 °C and from 7.41 °C to 15.36 °C, with an average of 11.80 °C. Daytime temperatures ranged from 17.05 °C to 28.91 °C, with an average of 22.43 °C, and from 17.36 °C to 27.34 °C, with an average of 22.18 °C. The relative humidity ranged from 58.22% to 70.9%, with an average of 65.52%, and from 56.32% to 68.18%, with an average of 64.25%. The soil at the experimental location underwent mechanical and chemical analysis (Table 1), according to Chapman and Pratt (1978).

Experiment design

The experiment's randomized complete block design required four replications. On November 15, in both seasons, flax seeds (from the Agricultural Research Centre, Giza, Egypt) incurred row planting, each 3.5 m long, with

Table 1. Analysis of the experimental soil (mechanical and chemical).

Seasons	Constant depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class
2020	00-30	40.69	44.61	10.69	3.9	Sandy
	30-60	38.21	43.01	13.79	4.9	Sandy
2021	00-30	38.72	42.60	13.69	5.0	Sandy
	30-60	36.49	38.11	17.79	7.59	Sandy

Seasons	Constant depth	Electrical conductivity		Saturation	Anions (milliequivalents liter^{-1})				Cations (milliequivalents liter^{-1})				$CaCo_3$ (%)	Organic matter
	(Cm)	pH	(dS mG^1)	(%)	CO_3G^2	HCO_3G	Cl	SO_4G^2	Ca++	Mg++	Na_G+	K+		(%)
2020	00-30	7.84	1.17	32	-	0.50	8.40	1.11	1.80	0.90	7.10	0.20	1.00	0.40
	30-60	7.89	1.79	27	-	0.60	8.00	1.40	2.10	1.50	6.20	0.20	6.00	0.07
2021	00-30	7.95	1.59	23	-	0.32	12.70	1.98	4.00	1.80	9.00	0.20	1.90	0.38
	30-60	7.85	1.81	25	-	0.45	15.40	2.15	5.60	2.00	10.20	0.20	1.30	0.32

Table 2. Water for Nubaria station's winter 2019/2020–2020/2021 flax crop growing phases.

Growth stages	Days (number)	Water for growth stage (m^3 ha^{-1})
Initial	20	326.04
Developing	30	1086.76
Mid	65	3260.27
Late	40	1141.08
Harvest	27	135.95
Total	182	5950

20 cm spacing between each row. The total area of the plot was 10.5 m^2 (3.0 m in width and 3.5 m in length).

Linum usitatissimum variety (Sakha-3) seeds sowing had a rate of 167 kg ha^{-1} using traditional agricultural methods. Applying 360 kg ha^{-1} of calcium superphosphate (15.5% P2O5) during pre-sowing transpired. Adding 180 kg ha^{-1} ammonium nitrate (33.5% N) in five equal doses and two equal doses of 120 kg ha^{-1} potassium sulfate (48.52% K2O) followed after germination. Irrigation occurs every five days. Cysteine at 0.0, 50, 75, and 100 mg L^{-1} and thiourea at 0.0, 200, 400, and 600 mg L^{-1} applications happened twice after 45 and 60 days from seed sowing.

Plant sample

Obtaining plant samples continued after 75 days of planting for morphological measurements and chemical analysis. Determining the seed yield and other yield-related traits ensued at harvest using random samples of 10 monitored plants from each plot.

Irrigation water requirements

Employing the Penman-Monteith equation and the crop coefficient (Allen *et al*., 1989) helped determine the required water for irrigation. The average amount of irrigation water utilizing the sprinkler irrigation systems for two seasons (2019–2020 and 2020–2021) was 5950 m^3 ha^{-1} (Table 2).

The following equation determined the irrigation water needed:

$$IWR = \frac{[Kr \times Kc \times ET0 \times I + LR] \times 4.2}{Ea}$$

Where:
IWR = Irrigation water requirement (m^3 ha^{-1}),
Kr = Reduction factor,
Kc = Crop coefficient,
ET_0 = Reference Evapotranspiration (mm day^{-1}),
I = Irrigation interval (day),
LR = Leaching requirement = 10% of the total water amount delivered to the treatment, and
Ea = Irrigation efficiency, 90%.

Table 3. Effect of different concentrations of cysteine or thiourea on morphological characters of flax (combined analysis of two seasons).

Items	Control	Cysteine (mg L^{-1})			Thiourea (mg L^{-1})		
		50	75	100	200	400	600
Plant height (cm)	58.67±0.58^{bc}	62.00±0.65^{b}	87.67±0.67^{a}	68.67±0.69^{b}	72.00±0.78^{a}	73.00±0.90^{a}	76.00±0.52^{a}
No. of Basel branches plant^{-1}	1.33±0.06^{b}	2.33±0.23^{a}	2.33±0.23^{a}	3.00±0.05^{a}	2.67±0.13^{a}	2.66±0.16^{a}	2.66±0.33^{a}
Shoot FW (g)	2.33±0.17^{f}	3.34±0.68^{e}	3.80±0.32^{d}	5.82±1.58^{b}	6.811.44±^{a}	5.20±0.87^{c}	5. 90±1.28^{b}
Shoot DW (g)	0.87±0.02^{c}	1.11±0.25^{bc}	1.25±0.20^{abc}	1.78±0.41^{ab}	2.03±0.42^{a}	1.67±0.30^{ab}	1.93±0.29^{ab}
Root FW (g)	0.21±0.00^{f}	0.44±0.11^{e}	0.65±0.10^{a}	0.55±0.16^{c}	0.51±0.15^{d}	0.45±0.08^{e}	0.62±0.12^{b}
Root DW (g)	0.12±0.02^{d}	0.18±0.05^{c}	0.35±0.06^{a}	0.22±0.04^{c}	0.20±0.04^{c}	0.21±0.04^{c}	0.24±0.04^{b}
Root length (cm)	8.33±0.33^{d}	9.67±0.58^{bcd}	12.00±0.73^{ab}	13.00±0.71^{a}	9.00± 0.58^{cd}	11.33± 0.67^{abc}	10.67± 0.33^{abcd}

a, b, c : Values in the same row with different superscripts significantly differ at $P < 0.05$.

Water content (WC%)

The water content calculation by Jin *et al.* (2017) used the following formula for estimating the leaf water content:

Water content (%) = (fresh weight − dry weight)/ Fresh weight × 100.

Physiological and biochemical studies

Indole acetic acid (IAA) content checking had the study employed the method described by Gusmiaty *et al.* (2019). Photosynthetic pigments (chlorophylls, carotenoids, lycopene, and B-carotene) evaluation used Nagata and Yamashita's (1992) method. Proline contents and total free amino acids assessment operated the technique of Tamayo and Bonjoch (2001). Total phenol and flavonoid content determination followed the system of Khatiwora *et al.* (2010). Discovering total anthocyanins engaged Ranganna's (1977) method. Superoxide dismutase (SOD, EC 1.12.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), peroxidase (POX, EC 1.11.1.7) activities, and reduced glutathione engaged the approach of Cao *et al.* (2004). The polyphenol oxidase activity (PPO, EC 1.10.3.1) incurred evaluation utilizing the Cho and Ahn (1999) technique. Radical scavenging activity (DPPH%; 2, 2'-Diphenyl-1- picrylhydrazyl) identification depended on the process of Liyana-Pathiranan and Shahidi (2005). Obtaining total soluble protein, total soluble sugars, carbohydrates, and oil contents continued, according to AOAC (1990). Fatty acid extraction followed the method outlined by Harbone (1984).

Statistical analysis

The data ran on a randomized complete block design statistical analysis. Duncan's multiple range test using SAS software version 9.2.0 compared means at a *P* value of 0.05 (Steel and Torrie, 1980). Data underwent principal component analysis (PCA) and Pearson correlation coefficient, according to Payne (2009), using the Genstat Pro software version 20th edition.

RESULTS

Morphological parameters

Data in Table 3 show the impact of cysteine and thiourea treatments on the growth traits of flax plants. Cysteine and thiourea as foliar applications significantly increased plant height, the number of basal branches per plant, shoot fresh and dry weights, root length, and root fresh and dry weights compared with the control. The maximum significant increase was visible in fresh and dry weights of the shoot and root at 600 mg L^{-1} thiourea.

Water contents (WC%)

The effect of cysteine or thiourea led to a significant increase in the flax WC% compared with the control (Figure 1). The lowest concentrations of thiourea statistically induced a significantly higher amount of WC% (12%) than the control.

Indole acetic acid (IAA)

The effect of cysteine or thiourea was to induce a significant increase in the IAA contents of flax leaves (Figure 1). The IAA content increased significantly by 73.35% in response to 50 mg L^{-1} cysteine and 47.37% in response to 600 mg L^{-1} thiourea compared with the control.

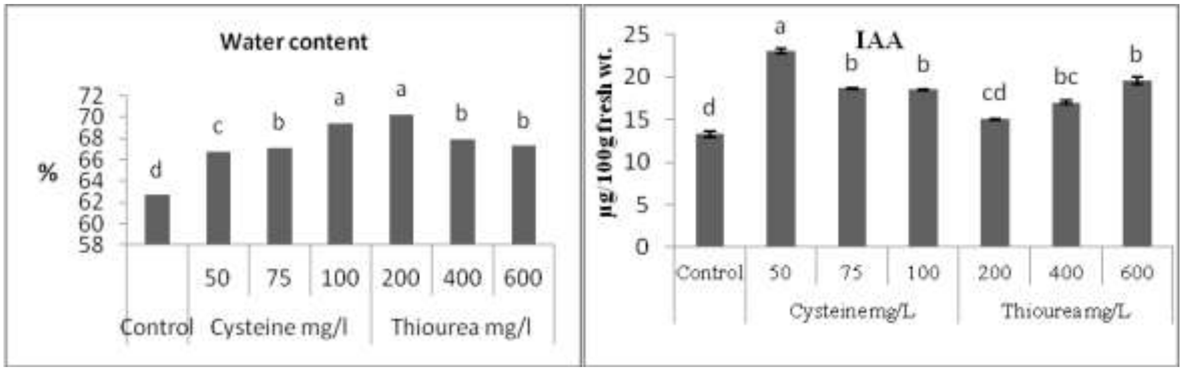

Figure 1. Effect of different concentrations of cysteine or thiourea on water content and IAA of flax leaves (a, b, c: Columns with different letters are significantly different at $P < 0.05$).

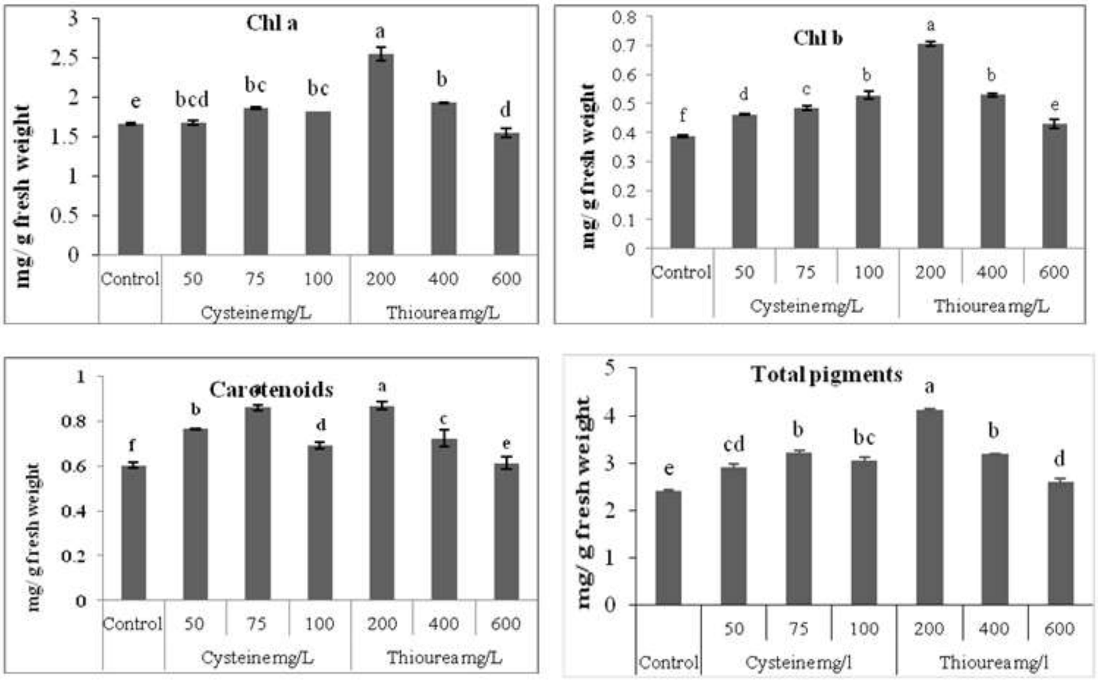

Figure 2. Effect of different concentrations of cysteine or thiourea on photosynthetic pigments of flax leaves (a, b, c: Columns with different letters are significantly different at $P < 0.05$).

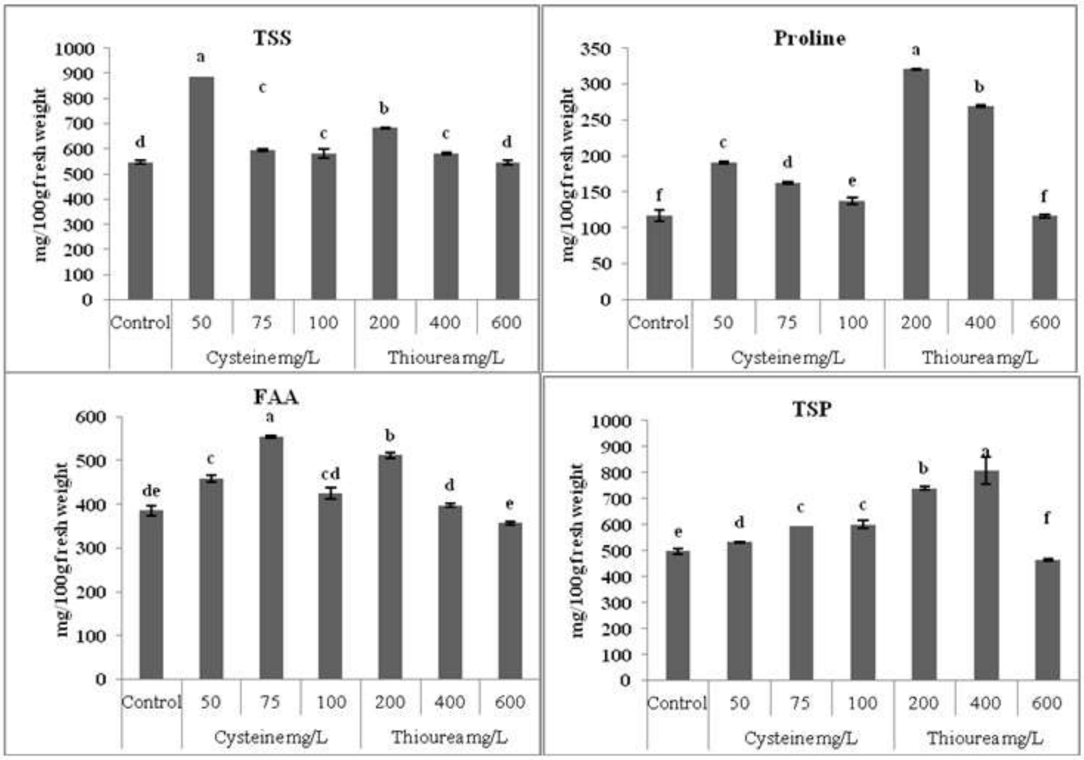

Figure 3. Effect of different concentrations of cysteine or thiourea on some organic solute of flax leaves (a, b, c: Columns with different letters are significantly different at $P < 0.05$).

Photosynthetic pigments

All concentrations of cysteine or thiourea considerably increased photosynthetic pigments (carotenoids, total pigments, chlorophyll a, and chlorophyll b) compared with the control, according to the data in Figure 2. A lower thiourea concentration achieved the highest increases in total pigments (55.42%).

Organic solutes

Data in Figure 3 showed that foliar application of cysteine or thiourea significantly augmented total soluble sugar and proline compared with its corresponding controls, except at 600 mg L^{-1} thiourea, providing a nonsignificant change. The highest rises in TSS appeared using the 50 mg L^{-1} cysteine and 200 mg L^{-1} thiourea. However, the maximum increase in Pro contents was evident at 200 mg L^{-1} thiourea.

Regarding free amino acids (FAA) and total soluble protein (TSP) contents, the treatments of cysteine or thiourea significantly increased them compared with the control, except at 600 mg L^{-1} thiourea, causing a significant decrease. The maximum increase in FAA manifested using 75 mg L^{-1} cysteine, followed by 200 mg L^{-1} thiourea, and the maximum upsurge in TSP was notable at 400 mg L^{-1} thiourea.

Antioxidant compounds

Treatment of the flax plant with cysteine or thiourea significantly increased phenolic and flavonoid contents versus the untreated plants, except for the thiourea (600 mg L^{-1}), which induced significant decreases in phenolic substances (Figure 4). The flavonoid contents rose (68.8% and 57.2%) with 400 mg L^{-1} thiourea and 75 mg L^{-1} cysteine, respectively.

The data presented in Figure 4 indicates that cysteine at a low concentration (50 mg L^{-1}) induced a nonsignificant increase in lycopene content but significantly decreased at

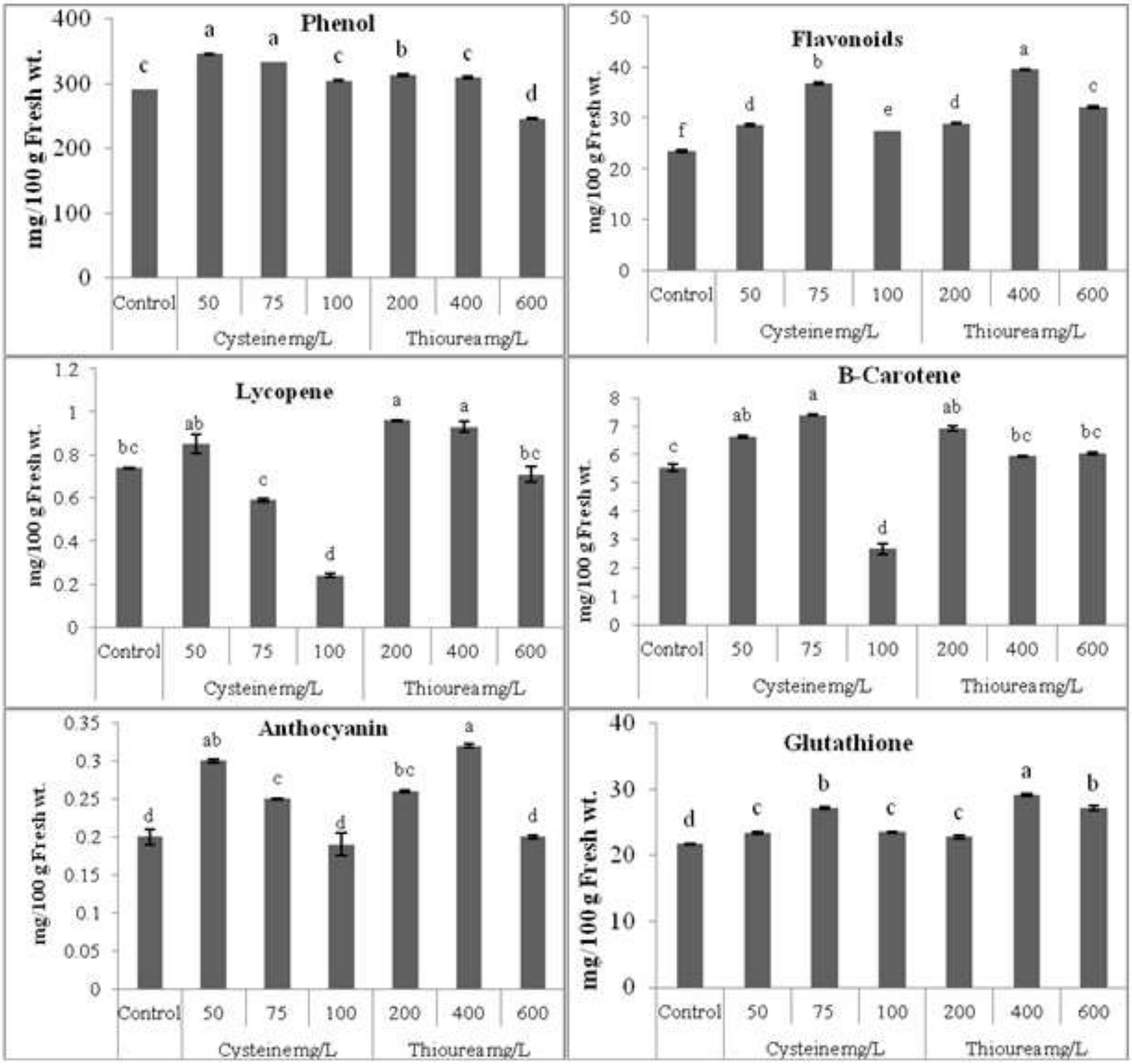

Figure 4. Effect of different concentrations of cysteine or thiourea on antioxidant compounds of flax leaves (a, b, c: Columns with different letters are significantly different at $P < 0.05$).

75 and 100 mg L^{-1} compared with the control. Treatment with thiourea substantially raised lycopene contents at 200 and 400 mg L^{-1} while inducing a nonsignificant decrease at 600 mg L^{-1} compared with the control.

Generally, various concentrations of cysteine or thiourea led to a significant increment in flax B-carotene contents versus the control, except at 400 and 600 mg L^{-1} thiourea, which induced nonsignificant increases, and 100 mg L^{-1} cysteine, causing a significant decrease. The highest values of B-carotene were evident with foliar sprays of cysteine at 75 mg L^{-1} and 200 mg L^{-1} thiourea over the untreated plants (Figure 4).

All applied treatments considerably boosted anthocyanin and glutathione contents compared with the control, except for 100 mg L^{-1} cysteine and 600 mg L^{-1} thiourea, inducing nonsignificant changes in anthocyanin contents. The maximum increment emerged in the case of 400 mg L^{-1} thiourea (Figure 4).

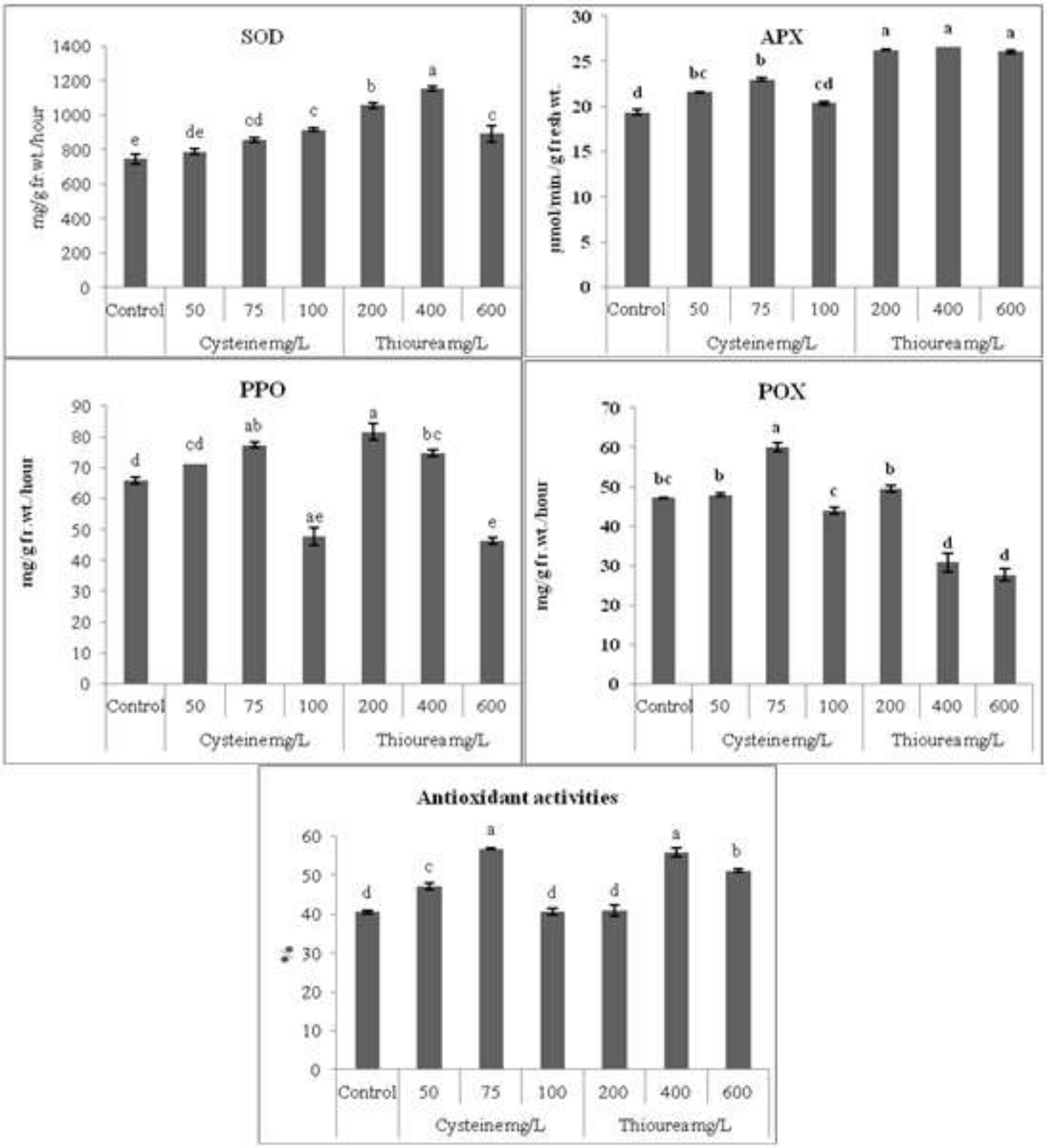

Figure 5. Effect of different concentrations of cysteine or thiourea on antioxidant enzymes and antioxidant activities (DPPH%) of flax leaves (a, b, c: Columns with different letters are significantly different at $P < 0.05$).

Antioxidant enzymes

Data in Figure 5 revealed that foliar application of cysteine and thiourea significantly enhanced superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities in flax leaves. The recorded highest increments occurred using 400 mg L^{-1} thiourea, compared with the control. The changes in polyphenol oxidase (PPO) activities caused gradual and significant increases in cases of 50 and 75 mg L^{-1} cysteine or 200 and 400 mg L^{-1} thiourea. Meanwhile, higher concentrations of both treatments markedly decreased the activity of PPO compared with the control. All treatments notably reduced POX activity, except at 75 mg L^{-1} cysteine and 200 mg L^{-1} thiourea, causing significant and nonsignificant increases,

Table 4. Impact of various concentrations of cysteine or thiourea on yield and its traits of flax plants (combined analysis of two seasons).

Items	Control	Cysteine (mg L^{-1})				Thiourea (mg L^{-1})	
		50	75	100	200	400	600
Plant height (cm)	68.33 ± 0.73^{e}	72.00 ± 1.03^{d}	87.66 ± 1.85^{a}	78.67 ± 1.19^{c}	82.00 ± 1.25^{b}	83.34 ± 1.40^{b}	86.67 ± 1.38^{a}
Technical stem length (cm)	59.33 ± 0.33^{c}	62.00 ± 1.53^{b}	65.66 ± 1.84^{a}	53.67 ± 0.67^{d}	67.57 ± 1.07^{a}	69.67 ± 1.45^{a}	53.67 ± 1.45^{d}
Fruiting zone length (cm)	9.00 ± 1.01^{e}	10.00 ± 0.67^{e}	12.00 ± 1.20^{d}	25.00 ± 1.53^{a}	14.33 ± 1.40^{c}	13.67 ± 1.02^{c}	17.00 ± 1.10^{b}
No. of fruiting branches plant^{-1}	4.33 ± 0.54^{c}	5.67 ± 0.33^{c}	4.33 ± 0.68^{c}	4.33 ± 0.20^{c}	5.00 ± 0.23^{c}	7.11 ± 0.16^{b}	8.67 ± 0.98^{a}
No. capsules plant^{-1}	6.67 ± 0.32^{e}	15.67 ± 0.30^{d}	17.33 ± 0.29^{d}	19.97 ± 0.31^{c}	32.00 ± 0.41^{a}	17.33 ± 0.12^{d}	28.33 ± 0.45^{b}
No. of seeds capsule^{-1}	9.67 ± 0.33^{a}	10.00 ± 1.15^{a}	9.33 ± 0.35^{a}	9.67 ± 1.08^{a}	9.67 ± 1.34^{a}	9.00 ± 1.56^{ab}	8.00 ± 1.33^{b}
Weight of 1000 seeds (g)	6.13 ± 0.13^{c}	8.27 ± 0.09^{b}	8.27 ± 0.30^{b}	7.60 ± 0.29^{b}	9.33 ± 0.33^{a}	8.00 ± 0.07^{b}	6.40 ± 0.43^{c}
Biological yield plant^{-1} (g)	7.02 ± 0.06^{bc}	9.98 ± 0.08^{ab}	9.09 ± 0.04^{ab}	8.11 ± 0.03^{b}	7.50 ± 0.02^{bc}	10.03 ± 0.06^{a}	7.62 ± 0.03^{bc}
Seed yield plant^{-1} (g)	0.73 ± 0.04^{c}	$1.06\ \pm0.03^{a}$	0.85 ± 0.02^{b}	0.90 ± 0.04^{b}	1.03 ± 0.05^{ab}	0.99 ± 0.02^{ab}	0.83 ± 0.02^{bc}
Straw yield plant^{-1} (g)	6.29 ± 1.04^{cd}	8.92 ± 0.95^{ab}	8.34 ± 0.87^{b}	7.27 ± 0.73^{c}	6.47 ± 0.99^{d}	9.24 ± 0.69^{a}	6.79 ± 0.77^{cd}
Biological yield (t ha^{-1})	6.23 ± 0.73^{d}	10.35 ± 0.83^{bc}	11.13 ± 0.39^{b}	11.353 ± 1.06^{b}	10.504 ± 1.17^{bc}	13.804 ± 1.00^{a}	13.320 ± 1.30^{a}
Straw yield (t ha^{-1})	5.35 ± 1.76^{d}	9.07 ± 1.65^{bc}	9.70 ± 1.09^{bc}	9.979 ± 1.65^{b}	9.179 ± 1.09^{bc}	12.152 ± 1.11^{a}	11.79 ± 1.20^{a}
Seed yield (kg ha^{-1})	878.23 ± 1.06^{e}	1276.63 ± 1.06^{d}	1427.69 ± 1.88^{c}	1373.26 ± 0.98^{c}	1325.04 ± 1.65^{cd}	1651.73 ± 1.69^{a}	1530.01 ± 1.87^{b}
Oil (%)	33.17 ± 0.18^{f}	36.24 ± 0.32^{d}	38.12 ± 0.14^{b}	36.81 ± 0.08^{c}	35.43 ± 0.18^{e}	39.00 ± 0.04^{a}	36.81 ± 0.03^{c}
Oil yield (kg ha^{-1})	291.31 ± 2.08^{f}	462.65 ± 2.76^{e}	544.24 ± 1.98^{c}	505.50 ± 1.68^{d}	469.46 ± 1.66^{e}	644.17 ± 3.09^{a}	563.20 ± 2.87^{b}
Carbohydrate (%)	25.19 ± 0.20^{d}	31.51 ± 0.72^{b}	34.18 ± 0.02^{a}	34.94 ± 0.45^{a}	28.64 ± 0.21^{c}	31.06 ± 0.20^{b}	28.77 ± 0.19^{c}

a, b, c : Values in the same row with different superscripts are significantly different at $P < 0.05$.

Table 5. Effect of different concentrations of cysteine or thiourea on the fatty acids profile of flax oil.

Fatty acids	Relative concentration (%)						
	Control	Cysteine (mg L^{-1})			Thiourea (mg L^{-1})		
		50	75	100	200	400	600
Myristic acid (C14:0)	2.85	---	---	---	---	---	---
Palmitic acid (C16:0)	6.56	6.97	6.93	6.44	6.92	6.71	6.71
Stearic acid (C18:0)	4.46	4.69	4.67	4.88	4.61	4.60	4.62
Oleic acid (C18:1c)(MUFA)	15.89	17.57	16.63	15.98	16.57	16.53	15.94
Elaidic acid (C18:1t) (MUFA)	0.83	---	---	0.82	0.89	0.86	0.85
*Linoleic acid (C18:2c) (PUFA)	13.71	14.95	15.21	14.31	15.16	14.42	13.69
Linolelaidic acid (C18:2t) (PUFA)	0.30	---	---	---	---	---	---
**Linolenic acid (C18:3n3) (PUFA)	53.02	55.81	56.56	56.37	55.85	56.89	58.19
γ- Linolenic acid (C18:3n6) (PUFA)	2.07	---	---	1.20	---	---	----
Total unsaturated	85.82	88.34	88.40	88.68	88.47	88.70	88.76
Total saturated	13.57	11.66	11.60	11.32	11.53	11.30	11.33
TUS/TS	6.32	7.58	7.62	7.83	7.67	7.85	7.83

respectively. Foliar application of cysteine or thiourea remarkably enriched the total antioxidant activities (DPPH), except for 100 mg L^{-1} cysteine and 200 mg L^{-1} thiourea, which induced a nonsignificant effect.

Yield and yield components

Data in Table 4 showed that cysteine and thiourea treatments caused an upsurge in yield parameters (plant height, technical stem length, fruiting zone length, the number of fruiting branches $plant^{-1}$, the number of capsules $plant^{-1}$, the number of seeds $plant^{-1}$, weight of 1000 seeds, biological yield $plant^{-1}$, seed yield $plant^{-1}$, straw yield $plant^{-1}$, and organic, straw, and seed yields). The highest increments in biological yield (t ha^{-1}; 121.36%), straw yield (t ha^{-1}; 126.8%), and seed yield (Kg ha^{-1}; 88.1%) were prominent in the case of 400 mg L^{-1} thiourea.

Nutritional content of seed yield

Oil and carbohydrate contents

Table 4 indicated that thiourea was superior to cysteine in significantly increasing oil% and yield (kg ha^{-1}). On the other hand, cysteine gave the highest significant carbohydrate % values in flax seeds**.**

Diversity in fatty acid profile

The fatty acids of the yielded flax seeds are prominent in Table 5. The most predominant saturated fatty acids were palmitic and stearic (6.56% and 4.46%, respectively) in the control plants, while the chief unsaturated fatty acids were oleic, linoleic, and linolenic acids (15.89%, 13.71%, and 53.02%, respectively). Treatment with cysteine and thiourea induced a marked decrease in total saturated fatty acids, accompanied by a significant increase in total unsaturated fatty acids. Linolenic and omega-3 levels rose with the tested treatments. Thiourea (600 mg L^{-1}) was the most effective treatment, giving the highest levels of total unsaturated fatty acids (88.76% on average) and markedly decreasing total saturated fatty acids (11.33% on average).

Principal Component Analysis (PCA) and Pearson Correlations

The PCA is a helpful data analysis and dimensionality reduction tool to transform data into a new coordinate system where the data's variance is optimal along the principal components (PCs) in linear combinations of the original variables. In this study, the first PC (PC1) explains the highest total variance (37.76%), and the second PC (PC2) explains the lowest significant amount of variance (17.35%). PC1 and PC2 demonstrated more than 55.11% of the total variance (Figure 6). Phenol, IAA, and root length (RL) indicated associations with cysteine at 50 and 100 mg L^{-1}, respectively. TSS, SPP, POX, and Pro showed nontreatment correlations. On the other side, most traits— FAA, lyco, Caro, antho, B caro, Chl a, Chl b, TSP, technical stem length (TZ), plant height (PH), SOD, seed yield $plant^{-1}$ (BWPP), 1000-SW, flav, APX, and PW—signify linkages with thiourea at 200 and 400 mg L^{-1} and cysteine (75 m gL^{-1}). The traits (GSH, seed yield $plant^{-1}$ [SWPP], root dry weight [RDW], Carb %, number of fruiting branches $plant^{-1}$ [NFBPP], DPPH, PH, shoot fresh weight [SFW], root fresh weight [RFW], shoot dry weight [SDW], number of basal branches $Plant^{-1}$ [NBPP], biological yield ha^{-1} [BYPH], fruiting zone length [FZ], seed yield ha^{-1} [SYPH], oil%, and oil yield ha^{-1} [OYPH]) had associations with thiourea at the rate of 600 mg L^{-1}. The data in Figure 7 represents the correlation coefficients between various traits and variables related to flax plants. Plant height moderately correlated positively with other variables, such as, NBPP, SFW, SDW, and RFW.

The NBPP positively correlated with several variables, e.g., SFW, SDW, RFW. SDW also showed strong positive correlations with SFW, RFW, RDW, and other variables. The RFW has positive correlations with RDW and RL. The RDW revealed correlations with RL and Chl a. Root length indicated a negative connection with Chl a, Chl b, Caro, and others. Longer roots may have linkages with lower values of variables—the chlorophyll (a or b) and carotenoid contents. Shoot fresh weight was positively correlated with Chl a, Chl b, and total pigments (TP). It also bore influences from

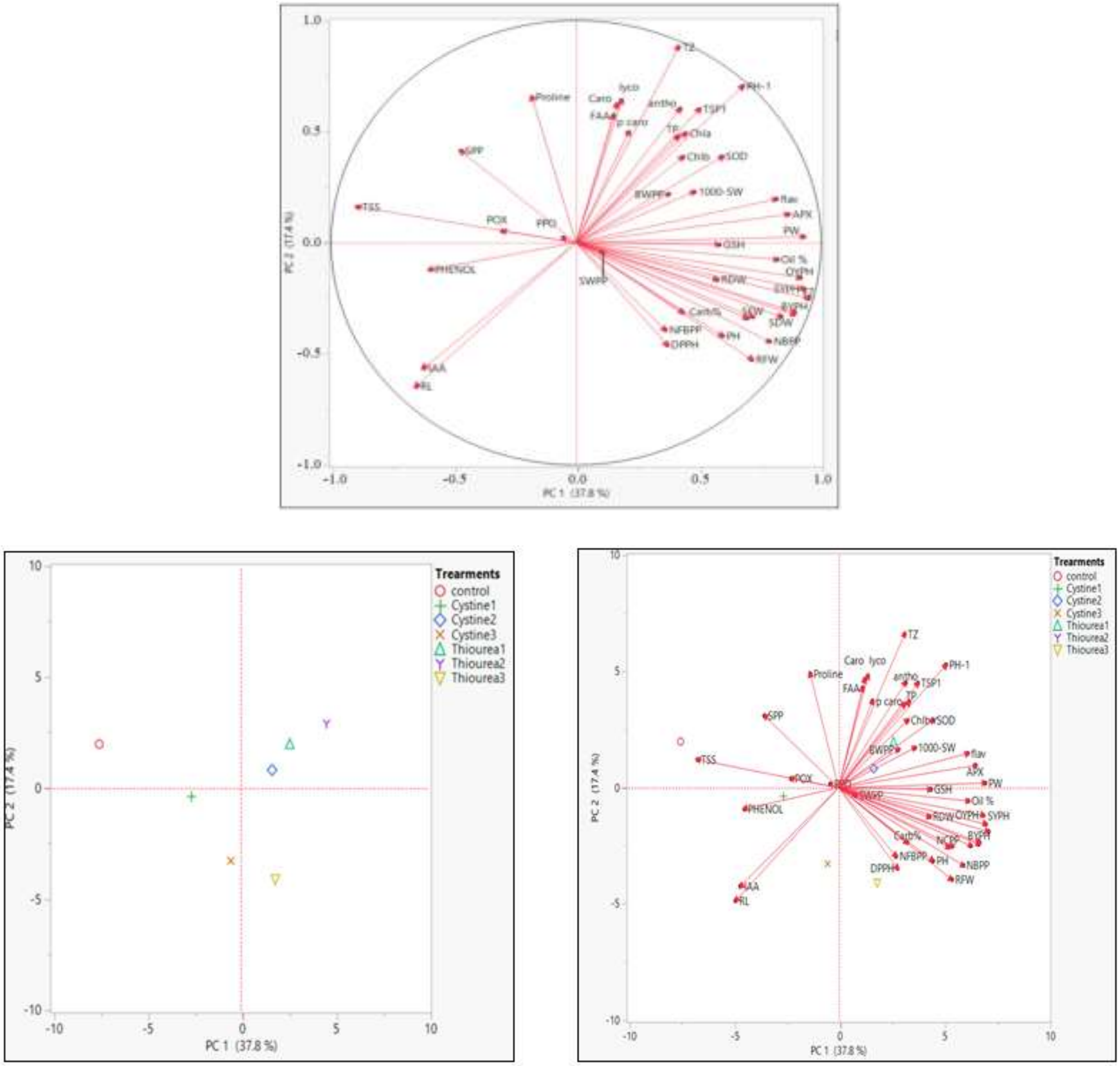

Figure 6. Principal component analysis (PCA) of growth, physiological, biochemical, and mineral traits in the investigated Cysteine (50, 75, and 100 mg L^{-1}) and Thiourea (200, 400, and 600 mg L^{-1}) treated and non-treated flax plants grown under sandy soil conditions. a) PCA loading plot of 43 measured traits, b) PCA score plot, and c PCA biplot.

these traits: PH, TZ, FZ, and BWPP. The NFBPP, NCPP, SPP, and SWPP variables also have mixed correlations, indicating that they may diversely influence the plant's 1000-SW, PW, BYPH, STYPH, SYPH, and OYPH. These variables had strong positive correlations, suggesting a high degree of interdependence. The variables POX, PPO, SOD, IAA, FAA, and DPPH have various correlations with potential relationships with different aspects of plant growth and physiology.

DISCUSSION

The enhanced effect of cysteine on the growth parameters of flax plants may be attributable to GSH synthesis (Künstler *et al.*, 2020).

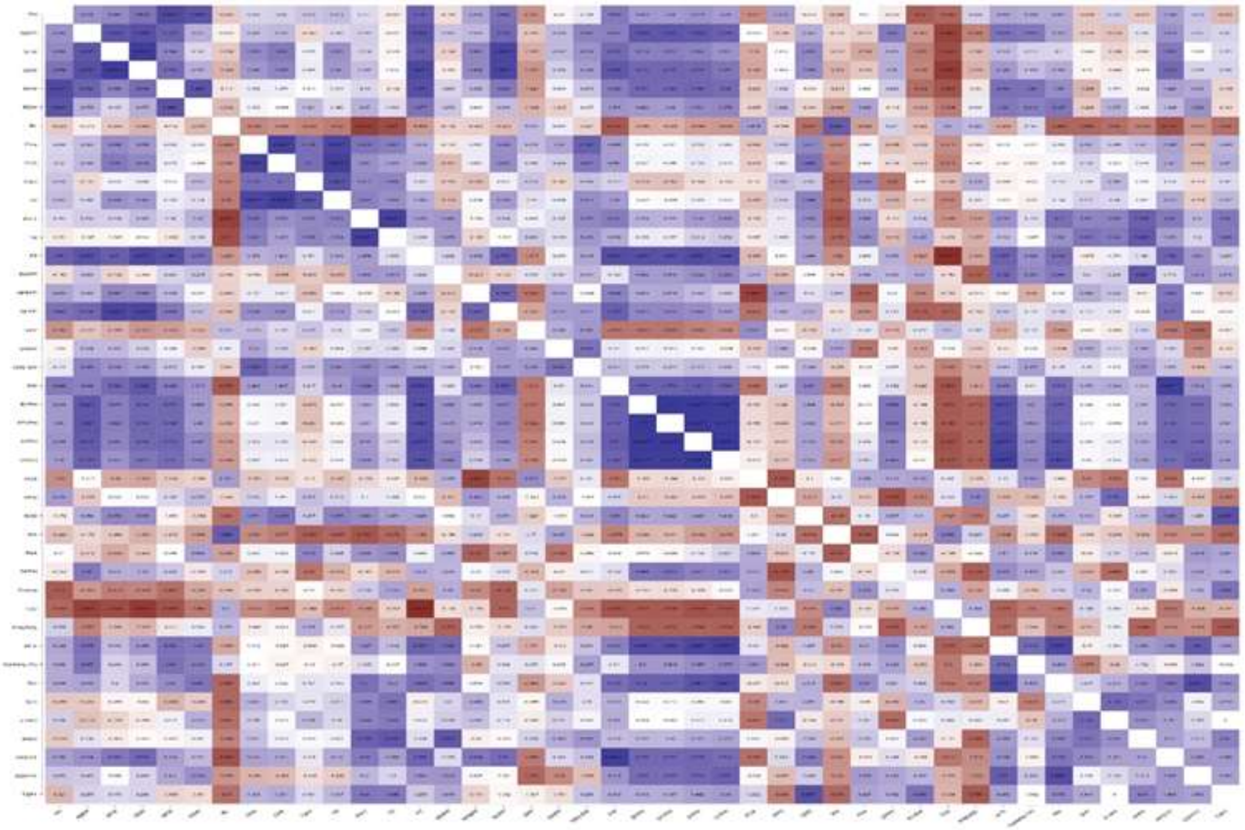

Figure 7. Heatmap of Pearson correlation analysis of all investigated traits in Cysteine and Thiourea treated and non-treated flax plants grown under sandy soil conditions. Blue and brown colors represent positive and negative correlations, respectively.

Meanwhile, thiourea could refer to its nitrogen-containing functional group and carbon, subsequently, growth-regulatory functions (Wahid *et al.*, 2017). Thiourea targeted the meristematic activity of apical tissues and stimulated cell division, resulting in greater shoot length and higher cell numbers, ultimately enhancing the fresh and dry weights of camelina's shoots and roots (Ahmad *et al.*, 2023). Furthermore, TU could contribute to cellular osmotic adjustment (Figure 3). Moreover, a rise in endogenous enzymatic activities (Figure 5) played a role in root growth, enriching plant development (Gins, 2022; Amangaliev *et al*., 2023).

The increase in growth rate accompanied by a rise in IAA content in shoot tissues suggests that cell proliferation and expansion may be positive (Abdallah *et al*., 2020). Interestingly, Zhao *et al*. (2014) found a strong correlation between sulfate availability and cellular L-cysteine and IAA levels. In *Brassica juncea* roots, TU priming can activate auxin metabolism to ensure increased IAA levels. It explains that it modulates the conversion of indole butyric acid (IBA) to indole acetic acid (IAA), as reported by Srivastava *et al*. (2017).

The rise in photosynthetic pigment levels (Figure 2) gained confirmation from Hussein and Alshammari (2022), who demonstrated that the sulfur resulting from cysteine exists in the electron transport system and may improve the rate of photosynthetic processes. Cysteine discovery acts as a signaling molecule, regulating and protecting the photosystems (Genisel *et al.*, 2015). This positive effect of cysteine on photosynthetic pigments may be because the chloroplast is the principal exporter of sulfide through sulfate reduction in the sulfur assimilation pathway (Hiji and Jerry, 2020). Waqas *et al.* (2019) found that applying TU increased photosynthetic pigments, likely due to its ability to enhance the efficiency of photosystems I and II and improve biomass production. Moreover, applying TU enhances photosynthetic rates via the phosphoenolpyruvate carboxylase pathway and phloem translocation, playing a role in photosynthates movement (Ahmad *et al.*, 2022).

A critical way that proline accumulates in plant tissues in response to various cysteine treatments is to reduce oxidative damage (Hussein and Alshammari, 2022). According to Elkelish *et al*. (2021), proline and total soluble sugar levels in wheat plants under stress from drought attained a boost with cysteine treatments, which could result from sulfur-donor molecules enhanced and involved in manufacturing basic organic composites. Improving osmolyte levels is essential for plant-cell-development tolerance in sandy soil; its accomplishment results from increasing the percentage of water content and the cytoplasmic osmotic pressure (Figures 1 and 3). Ahmad *et al*. (2022) discovered that, in addition to controlling the synthesis of osmoprotectants (FAA, proline, and glycine betaine and soluble sugars), applying TU favored plant-water relations**.**

The observed increase in antioxidant compounds by foliar spraying of cysteine and thiourea can refer to either enhanced biosynthesis or reduced degradation of these compounds. Cysteine or thiourea applications significantly raised plant phenolic content (Hussein and Alshammari, 2022; Waqas *et al.*, 2019). Lycopene serves as a precursor to β-carotene, a fat-soluble carotenoid that boosts antioxidant activity approximately twice as potent as that of β-carotene itself and also plays essential roles in cell signaling and communication processes (Maoka, 2019). Additionally, carotenoids have close links to photosynthesis, functioning as integral components of the light-harvesting system. Hence, in the presented experiment, the elevation in lycopene and β-carotene levels can be due to improved photosynthetic processes (Figures 2 and 4). The notable rise in glutathione due to cysteine or thiourea treatments may refer to cysteine as a precursor to vital biomolecules like glutathione, acting as an antioxidant (Künstler *et al.*, 2020). Baqer *et al.* (2020) found that applying thiourea improved wheat plants' phenols, riboflavin, anthocyanins, and ascorbic acid synthesis.

The tested materials caused a gradual increase in antioxidant enzyme activity (Figure 5). The capacity for scavenging free radicals (ROS), denoted as DPPH%, significantly mitigates heightened free radicals responsible for oxidative harm in plant cells. Abdallah *et al.* (2020) showed that elevated levels of antioxidant enzymes can indicate a response to escalated ROS production. The increases in antioxidant enzymes due to cysteine treatments align with Hiji and Jerry's (2020) study results. Nasibi *et al.* (2016) stated that cysteine reduced the influence of salinity on wheat plants by reducing H_2O_2 and MDA contents through the actions of antioxidant enzymes. Additionally, the function of the cysteine thiol groups often exists at the active positions and is a modulator for enzymatic reactions (Richau *et al.*, 2012)**.** Waqas *et al.* (2019) on various crop plants and Ahmad *et al.* (2022) on canola found that thiourea led to significant increments in the SOD and catalase enzymes activities, accompanied by a substantial decrease in the activities of peroxidase.

Higher levels of photosynthetic pigments (Figure 2) and better vegetative development (Table 3) may be the causes of the observed increase in flax production. Consequently, there may be a rise in the synthesis of photoassimilates carried to the reproductive organs as seeds. As an osmoprotectant agent, thiourea controls source-to-sink connections to enhance plant quality and agricultural productivity (Wahid *et al.*, 2017).

The data presented in Table 4 revealed that thiourea was superior to cysteine in substantially increasing seed yield, oil%, and oil yield. Meanwhile, cysteine gave the most significant rates of carbohydrate (%) in flax seeds. In this connection, Ahmad *et al.* (2023)

on camelina and Harisha *et al.* (2023) on chia plants observed that the treatment of thiourea increased the oil content and seed oil quality under stress conditions via moderating the plant-water relations, regulation of soluble sugars, and stimulation of the antioxidant enzyme activities. Pandey *et al.* (2013) demonstrated that the association between TU application and seed oil concentration could refer to the production of acetyl coenzyme A (a precursor for synthesizing long-chain fatty acids). This process improves seed oil content, leading to a high oil yield.

Applying cysteine and thiourea to flax increased the unsaturated/saturated fatty acid ratio in the seed yield of treated plants. Notably, the treatments led to an increase in polyunsaturated fatty acids (specifically linolenic and omega-3). These findings align closely with outcomes from studies by Bakry *et al.* (2012), who stated that applying amino acids to sunflower plants led to a rise in unsaturated fatty acid content, which increased omega-3 PUFA. In the same field, Ahmad *et al.* (2023) demonstrated that applying thiourea enhanced the levels of linoleic and linolenic acids (unsaturated fatty acids) while reducing the level of palmitic and stearic acid (saturated fatty acids) in various camelina genotypes. Moreover, Harisha *et al.* (2023) reported that thiourea preserved seed oil quality in chia plants, particularly concerning linolenic acid and polyunsaturated fatty acid contents, even when exposed to drought stress.

CONCLUSIONS

Sandy soil negatively impacted the growth and yield parameters of flax plants. The results gained herein revealed the possibility of easing such effects using cysteine and thiourea, which enhanced plant water content, synthesized photosynthetic pigments, and upregulated osmoprotectants, antioxidant enzyme activities, and antioxidant compounds expected to improve plant growth and yield. Cysteine or thiourea application effectively enhanced seed oil content with increased levels of unsaturated fatty acids and a reduction in saturated fatty acids, revealing the improvement in the nutritional value of seed oil. Thiourea treatment at 400 mg L^{-1}, followed by 75 mg L^{-1} cysteine, was most evident in increasing most studied parameters. The results of this article are applicable on a large scale in the newly reclaimed sandy soil areas that afford sustainability in farming methods and safer environmental practices.

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