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Response of sesame (*Sesamum indicum* L.) to foliar-applied thiourea under saline conditions

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Abstract
Salt stress is among the most pervasive limitations for food production, which eventually results in negative financial, environmental and social consequences. Thiourea is a vital chemical which improves growth and yield of plants. Sesame (*Sesamum indicum* L.) is a crop of semi-arid and arid regions, whose medicinal and seed oil properties are well known. A pot experiment was carried out to examine the impact of thiourea on sesame plants exposed to saline conditions. Two cultivars of sesame, i.e., TH-5 and TH-6 were used. Two levels (0 and 70 mM) of salinity stress as well as two foliar-applied levels (water spray and 150 mM) of thiourea were maintained after 28 and 34 days of seed sowing, respectively. Data for various morphological and physiological attributes was taken after 56 days of seed sowing. The results showed that salinity stress diminished shoot mass along with root mass (fresh and dry) as well as shoot and root lengths. Of physiological parameters, chlorophyll and gas exchange attributes were also negatively affected by the salinity stress. Salinity stress escalated the activities of SOD and POD as well as the levels of MDA. Foliar-applied thiourea raised the contents of chlorophyll b, total chlorophyll and carotenoids of cv. TH-6 under salt stress. The activity of POD of both cultivars under control and salt stress conditions and root K^+ concentration of cv.TH-5 under salt stress were also enhanced by thiourea. Overall, foliar application of thiourea mitigated the negative impacts of salinity on sesame.

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Introduction

Sesame (*Sesamum indicum* L.) is amongst the primitive crops of oilseed importance. It is being extensively grown in Africa and Asia (Dossa et al., 2017). Sesame delivers one of highest as well as richest edible oils (Pathak et al., 2014) with low levels of saturated fatty acids (Sankar et al., 2005). The seed contains 50–60% oil comprising antioxidants of natural type like sesamolin, sesamol and sesamin (Anilakumar et al., 2010). The sesame oil antioxidants have health promoting impacts like lessening hypertension and levels of cholesterol (Sankar et al., 2005), and neuroprotective impacts against the brain impairment (Cheng et al., 2006). Sesame plant has varied rhizosphere films that aid the crop to develop better with the help of extensive stem; its roots are very much expanded in the soil or sand and are highly flexible to harsh conditions (Golestani and Pakniyat, 2007).

Salinity is one of the most distressing environmental aspects (Shrivastava and Kumar, 2015) and the agricultural land disturbed by it is increasing internationally due to natural phenomena as well as agricultural practices (Munns and Tester, 2008). Salinity stress restricts productivity of plants (Paul, 2012),

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seed germination and seedling development (Farooq et al., 2015), and vegetative as well as reproductive growth (Bano and Fatima, 2009). Salt stress also has impacts on photosynthesis primarily by diminution in chlorophyll content, leaf area and stomatal conductance as well as by a decline in efficiency of photosystem II (Netondo et al., 2004). Threats which plants face during salt stress include ionic and osmotic stresses (Flower and Colmer, 2008), besides oxidative stress (Parida and Das, 2005). A surplus amount of soluble salts in the soil lowers down osmotic potential perturbing the uptake of nutrients along with water, thus creating ionic imbalance as well as toxic conditions in plants. All these aspects cause deterioration in various biochemical along with physiological processes which ultimately cause diminished growth of plants (Hamdia and Shaddad, 2010).

The capability of the plant to tolerate a stress can be enhanced by external application of stress relieving chemicals (Farooq et al., 2009). Thiourea, also chemically named as Thiocarbamide, is a nitrogen- and sulfur-containing compound (Wahid et al., 2017). It is of vital importance among these chemicals, being highly soluble in water as well as effortlessly absorbed by the living tissues (Gul and Weber, 1998). Thiourea (TU) has been identified as an effectual bio-regulator enabling the crops to develop stress tolerance (Shrivastava et al., 2008). Its application is believed to have a considerable role in diminishing damage because of oxidative stress as well as processes associated with it (Zahra et al., 2018). Thiourea is potent in improving plant development and growth under salinity, heat stress, drought and heavy metal toxicity. It improves nutrient acquisition by the roots and their assimilation as well as leaf gas exchange at physiological level, whereas protein biosynthesis and sugar metabolism at biochemical level (Wahid et al., 2017).

The hypothesis of present study was that thiourea may protect the plants of sesame from injurious effects of salt stress. Thus, the current study objective was to assess the role of thiourea in alleviating salt stress induced impacts on sesame plants.

Materials and Methods

An experiment was conducted in the old botanical garden of the University of Agriculture, Faisalabad, Pakistan to evaluate the performance of two sesame cultivars (TH-5 and TH-6) under saline conditions and foliar application of thiourea. The seeds of both cultivars were taken from the Ayub Agricultural Research Institute, Faisalabad. Seed sowing was done in a manner that every pot consisting of sand was provided with fifteen seeds. Five seedlings were allowed to grow in every pot for a few days after germination. Every pot was watered with 250 mL of water daily. Hoagland nutrient solution was supplied to the plants every week as a nutrient source until seedlings were fifteen day-old. After four weeks of seedling growth, salt stress (NaCl) was applied in Hoagland nutrient solution, while the control plants were treated with salt free Hoagland solution. The salt solution used in this study was of two different concentrations, 0 and 70 mM NaCl. When sesame plants were 34 day-old, foliar application of thiourea was applied at two different levels: water spray and 150 mM. Each treatment had four replications. Gas exchange and morphological parameters were recorded after 56 days of sowing. Physiological and biochemical variations were also documented. At crop maturity, yield attributes were also recorded.

Morphological attributes

Morphological attributes included root and shoot fresh and dry weights along with root and shoot lengths that were recorded after 56 days of sowing.

Physiological attributes

Carotenoids along with chlorophyll *a* and *b* were measured following Arnon (1949). Readings were taken on a spectrophotometer at three different wavelengths (480, 645 and 663 nm). Stomatal conductance, sub-stomatal CO_2 concentration, transpiration, and photosynthetic rate were measured from a fresh fully developed leaf from each plant using an infra-red gas analyzer. Ion (K^+, Ca^{2+} and Na^+) analysis was done using a flame photometer.

Biochemical attributes

For evaluation of oxidative stress in sesame plants, malonaldehyde (MDA) was determined using a spectrophotometer. To check the potential and performance of plants against oxidative stress under different conditions imposed to them, the activities of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) were measured. To determine MDA content, 0.25 g of leaf was ground in 5 mL of 5% TCA (trichloroacetic acid), then centrifuged at 12000 *g* for15 minutes in a centrifuge machine. In a test tube, 500 µL of supernatant were taken and then mixed with 500 µL of 2-thiobarbituric acid (0.5%) in TCA solution (20%). After that, the test tubes were placed in a water bath at 100 °C for one hour, kept for 10 minutes in ice, and then centrifuged again at 10,000 *g* for10 minutes. Then the filtrate was taken in a cuvette and reading was taken at the wavelengths of 532 nm and 600 nm on a spectrophotometer. For the estimation of activities of antioxidants, 0.25 g of the leaf sample was ground in potassium phosphate buffer (50 mM and pH=7.8). SOD activity determination was done by mixing 400 µL of distilled H_2O, 250 µL of potassium phosphate buffer, 100 µL of L-methionine, 100 µL of triton, 50 µL of NBT, 50 µL sample and 50 µL riboflavin. This mixture was then kept under light for about 15 minutes and then reading was taken at the wavelength of 560 nm on a spectrophotometer. Determination of CAT activity was done by mixing 1.9 mL of potassium phosphate buffer, 1 mL of 5.9 mM H_2O_2 as well as 0.1 mL of the enzyme extract. The reading was then taken at 240 nm with intervals of 30 seconds on a spectrophotometer. Determination of POD activity was done by mixing 40 mM of H_2O_2, 20 mM of guaiacol, 50 µL enzyme extract and 750 µL potassium phosphate buffer, and then the reading was taken at a wavelength of 470 nm at intervals of 30 seconds on a spectrophotometer.

ANOVA (analysis of variance) was applied by using Co-STAT software for statistical analysis. The experiment was arranged in a completely randomized design having four replicates of each treatment.

Results

Salinity stress significantly diminished the fresh as well as dry weights of shoot of the two cultivars of *Sesamum indicum* (TH-5 and TH-6). Thiourea (TU) foliar application did not improve these attributes. However, cultivar TH-6 performed better than TH-5 under salt stress (**Figure 1**; **Table 1**).

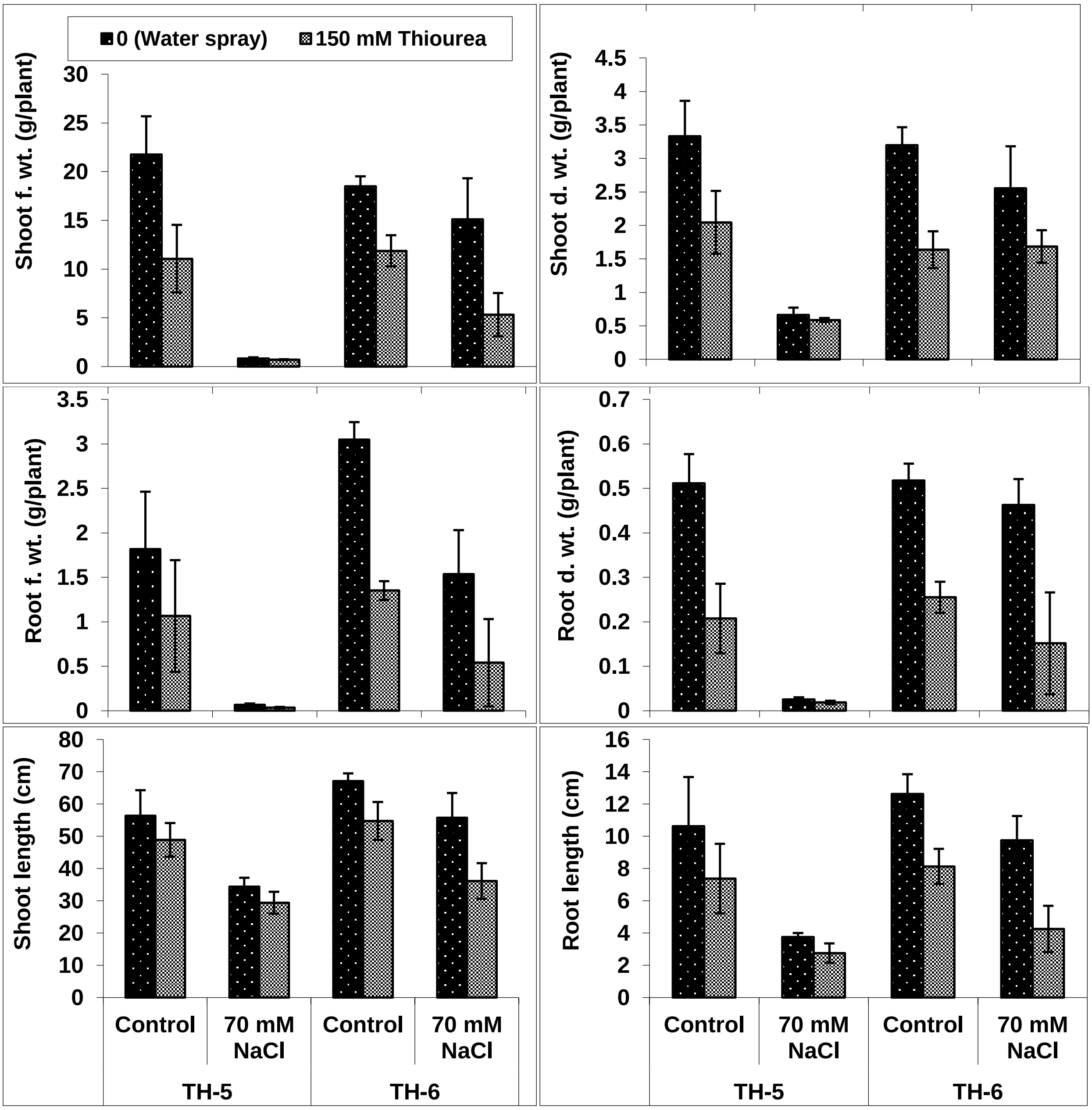

Figure 1. Morphological variations in non-stressed and salt-stressed sesame plants treated with foliar-applied thiourea.

Table 1. Analysis of variance for different parameters of non-stressed and salt-stressed sesame plants treated with foliar application of thiourea.

SoV	df	SFW	SDW	RFW	RDW	Shoot length	Root length
Cultivars (C)	1	135.383*	3.001*	6.103**	0.194**	1001.281**	52.531*
Salinity (S)	1	850.162***	11.158***	13.036***	0.347***	2556.125***	166.531***
Tiourea (T)	1	371.417**	7.201**	6.0334**	0.390***	990.125**	101.531**
C x S	1	228.124**	6.233**	0.105ns	0.133**	66.125ns	11.281ns
C x T	1	15.68ns	0.567ns	1.821ns	0.034ns	190.125ns	16.531ns
S x T	1	27.6768ns	1.806ns	1.009ns	0.030ns	11.281ns	0.781ns
C x S x T	1	94.462ns	0.134ns	1.757ns	0.059ns	47.531ns	5.281ns
Error	24	26.803	0.555	0.675	0.014	119.192	10.697
SoV	**df**	**Chl. *a***	**Chl. *b***	**Total Chl**	**Carotenoids**	***A***	***E***
Cultivars (C)	1	1.531ns	6.204ns	0.001ns	0.180ns	41.632***	0.585*
Salinity (S)	1	0.064*	0.003ns	0.040ns	0.018ns	25.063**	1.181**
Tiourea (T)	1	0.003ns	0.009ns	0.001ns	0.016ns	93.639***	0.073ns
C x S	1	0.003ns	0.038ns	0.019ns	0.136ns	3.934ns	0.065ns
C x T	1	0.024ns	0.039ns	0.125ns	0.037ns	280.134***	2.743***
S x T	1	0.004ns	0.033ns	0.060ns	0.215*	35.574**	1.838***
C x S x T	1	0.360***	0.098*	0.834***	0.737***	1.548ns	2.531ns
Error	24	0.012	0.017	0.834	0.045	2.646	0.119
SoV	**df**	***A/E***	**C_i/C_a**	**g_s**	**C_i**	**Shoot Na^+**	**Root Na^+**
Cultivars (C)	1	0.064ns	0.020*	8128.125***	2559.164*	9.031ns	38.28125***
Salinity (S)	1	1.941ns	4.5e-6ns	6903.125***	0.458ns	242.0***	282.03125***
Tiourea (T)	1	13.629**	0.045**	1653.125*	5532.731**	1.125ns	10.125***
C x S	1	1.384ns	0.043**	1653.125*	5386.441**	19.531ns	60.5***
C x T	1	6.962*	0.036**	39903.125***	4613.521**	38.281*	30.031***
S x T	1	2.515ns	0.001ns	10153.125***	171.912ns	72.0**	94.53***
C x S x T	1	0.201ns	0.021*	903.125ns	2686.994*	132.031***	36.125***
Error	24	1.580	0.004	246.875	502.358	7.791	0.71875
SoV	**df**	**Shoot K^+**	**Root K^+**	**Shoot Ca^{2+}**	**Root Ca^{2+}**	**MDA**	**SOD**
Cultivars (C)	1	18.757***	7.031***	21.125***	6.125**	171.009**	1166.324ns
Salinity (S)	1	99.757***	40.5***	78.125***	84.5***	112.984*	5372.438***
Tiourea (T)	1	14.445***	7.031***	22.781***	30.03125***	151.445*	42.113ns
C x S	1	2.820ns	12.5***	2.531ns	3.125*	636.326***	261.918ns
C x T	1	17.257***	2.53**	8.0**	16.53***	888.216***	581.831ns
S x T	1	18.757***	8.0***	28.125***	9.031***	215.016**	2827.332**
C x S x T	1	15.820***	10.12***	9.031**	16.53***	834.984***	1830.881*
Error	24	0.888	0.281	0.760	0.5	20.299	299.320
SoV	**df**	**CAT**	**POD**				
Cultivars (C)	1	231200**	8.51***				
Salinity (S)	1	35915ns	1.89***				
Tiourea (T)	1	35912ns	5.42***				
C x S	1	57800ns	3.27ns				
C x T	1	49928ns	1.24ns				
S x T	1	800ns	1.08ns				
C x S x T	1	3200ns	1.63ns				
Error	24	29245.3	1.71				

*, **, *** = significant at 0.05, 0.01, 0.001 levels, respectively; ns = non-significant, SFW = Shoot fresh weight, SDW = Shoot dry weight, RFW = Root fresh weight, RDW = Root dry weight, Chl. *a* = Chlorophyll *a*, Chl. *b*= Chlorophyll *b*, Total Chl. = Total Chlorophyll, *A* = Net CO_2 assimilation rate, *E* = Transpiration rate, *A/E* = Water use efficiency, g_s = Stomatal conductance, C_i = Sub-stomatal CO_2 concentration, MDA = Malondialdehyde, SOD = Superoxide dismutase, CAT = Catalase, POD = Peroxidise

Salinity imposition significantly declined the root fresh weight along with dry weight of both sesame cultivars. Exogenously applied thiourea did not increase these parameters. Cultivar TH-6 performed better than TH-5 under salinity conditions (**Figure 1**; **Table 1**).

Salinity stress imposition significantly reduced the shoot length besides root length of the two sesame cultivars. Thiourea application brought about a significant diminution in the shoot as well as root length. Cultivars differed significantly for both parameters. However, TH-6 performed better under non-stressed as well as stressed conditions (**Figure 1**; **Table 1**).

Salinity brought about a significant decrease in chlorophyll *a* of cultivar TH-6, while no such decrease was observed in cultivar TH-5. Thiourea given through foliar application improved the contents of chlorophyll *b* of cultivar TH-6 under salt stress. Total chlorophyll was also improved through exogenous application of thiourea of cultivar TH-6, but not of cultivar TH-5. Thiourea foliar application brought about a significant increase in carotenoid content of the cultivar TH-6. Cultivars differed significantly under thiourea application and salinity imposition (**Figure 2**; **Table 1**).

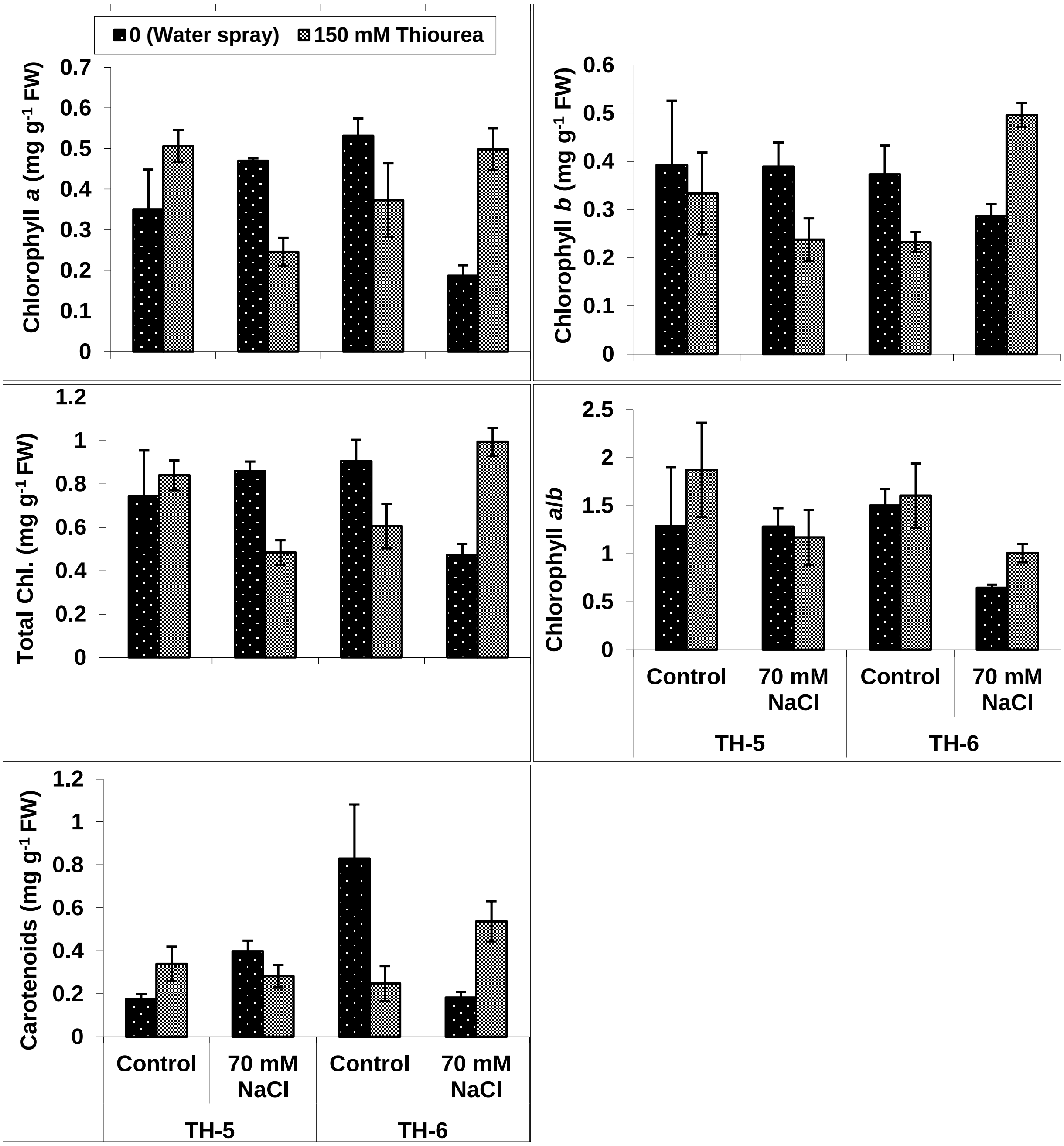

Figure 2: Photosynthetic pigments of non-stressed and salt-stressed sesame plants treated with foliar-applied thiourea.

The two cultivars differed significantly for net CO_2 assimilation rate (A). Cultivar TH-6 showed high net CO_2 assimilation rate (*A*) as compared to that of cultivar TH-6. Foliar-applied thiourea did not significantly improve this parameter but brought about a decline in it. The two sesame cultivars also differed significantly for the transpiration rate (*E*). Cultivar TH-6 showed high transpiration rate than did cultivar TH-5 under the foliar application of TU. Salinity significantly declined transpiration rate of the sesame plants. Salinity had a non-significant impact on water use efficiency (*A*/*E*) of the sesame plants. Thiourea application significantly elevated water use efficiency of the sesame plants. Salinity caused a non-significant impact on relative internal CO_2 concentration (C_i/C_a) of the sesame cultivars, whereas external application of thiourea escalated relative internal CO_2 concentration (C_i/C_a) of the cultivar TH-5. Stomatal conductance (g_s) of the sesame cultivars decreased significantly by the salt application. Foliar-applied thiourea significantly increased the stomatal conductance (g_s) of cultivar TH-6 under stressed as well as non-stressed conditions. The two sesame cultivars differed significantly under exogenous application of thiourea. Cultivars differed significantly for sub-stomatal CO_2 concentration (C_i); cultivar TH-5 performed better than TH-6 upon the external application of thiourea under salinity stress (**Figure 3**; **Table 1**).

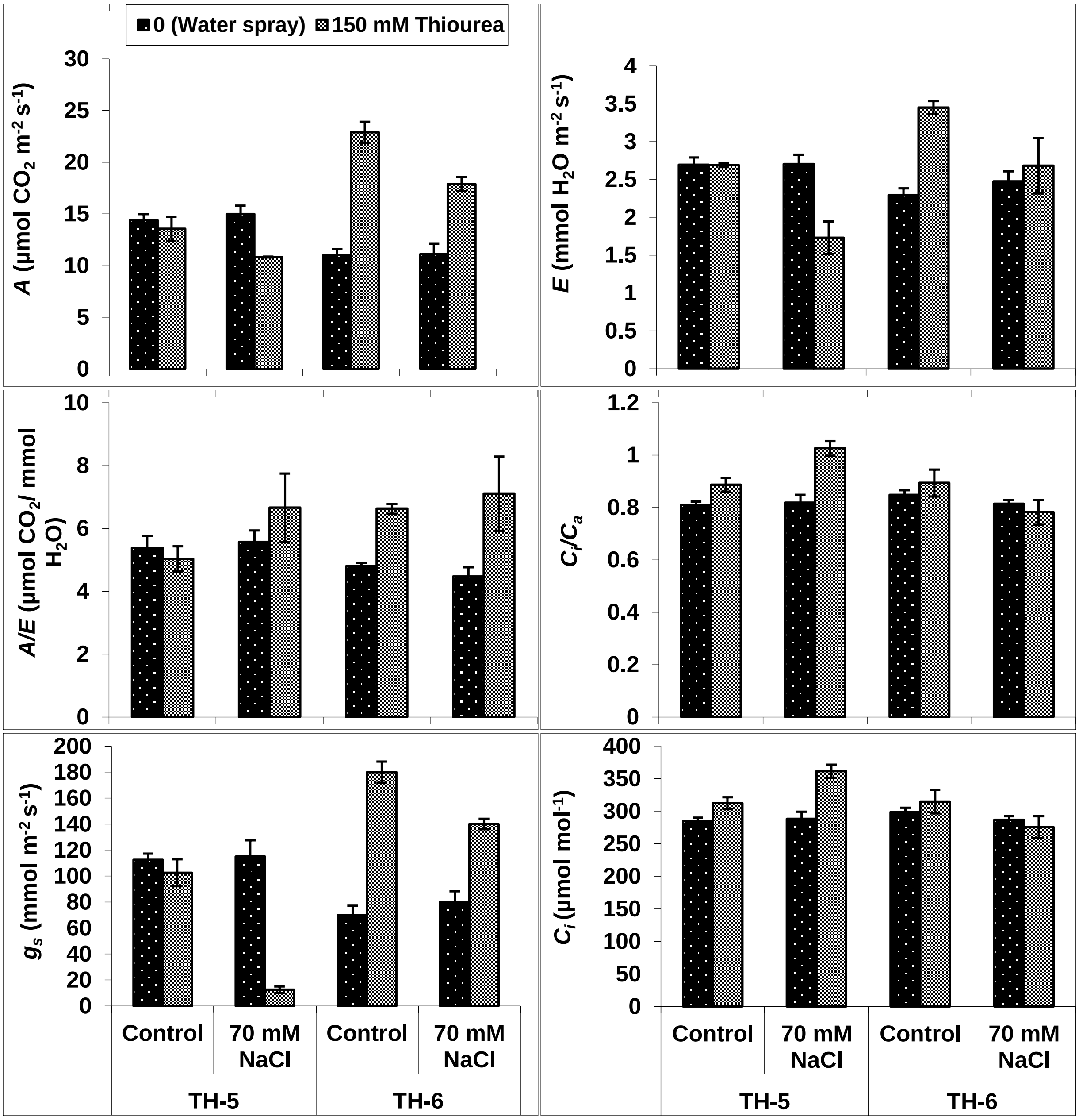

Figure 3. Gas exchange properties: Net CO_2 assimilation rate (*A*), transpiration rate (*E*), water use efficiency (*A/E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) of non-stressed and salt-stressed sesame plants treated with foliar-applied thiourea.

Salt stress significantly boosted the shoot Na^+ of the sesame plants. However, the two cultivars differed significantly upon the application of thiourea. Cultivar TH-6 showed lesser shoot Na^+ content as compared to that of cultivar TH-5 upon foliar application of TU under salty environment. Salinity had a significant escalating effect upon the root Na^+ of both sesame cultivars. The two cultivars differed significantly for root Na^+. Foliar-applied TU significantly escalated root Na^+ of the sesame cultivars in the presence of salinity. However, TH-5 showed lesser root Na^+ content in the presence of salt stress under water spray (**Figure 4**; **Table 1**).

Salinity exhibited a significant decreasing impact on shoot K^+ of TH-5 under both water and TU spray and TH-6 under water spray. External application of TU significantly reduced shoot K^+ of TH-5 under salt regime in comparison with the controls. Foliar-applied TU did not show a significant impact upon shoot K^+ of cultivar TH-6 under salt stress. Salt application significantly enhanced the root K^+ content of cultivar TH-6. TH-5 exhibited a significant rise in root K^+ content of the plants treated with salinity and TU. TH-6 also exhibited a significant increment in root K^+ in presence of salt stress under both water and TU spray (**Figure 4**; **Table 1**).

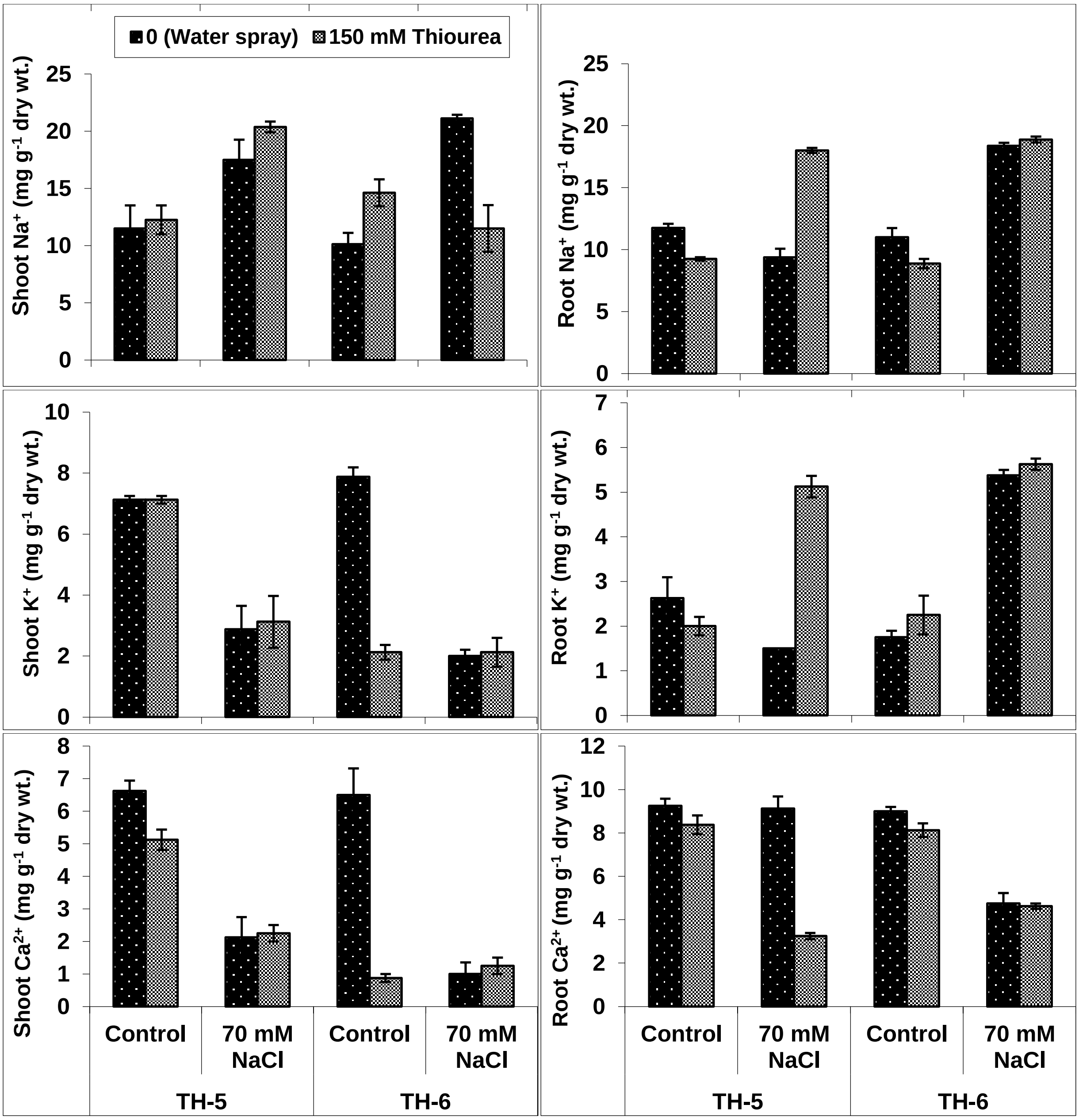

Figure 4. Mineral nutrients in non-stressed and salt-stressed sesame plants treated with foliar-applied thiourea.

Shoot Ca^{2+} of cultivar TH-5 decreased significantly upon salt application, while it decreased in cv. TH-6 under water spray. Foliar-applied TU exhibited a significant decrease in shoot Ca^{2+} content of cultivar TH-5 in the presence of salinity stress in comparison with the controls and a significant decrease in TH-6 plants under the control conditions. Salinity stress significantly declined root Ca^{2+} of TH-6. TU application significantly declined the root Ca^{2+} of both cultivars in the presence of salinity stress compared with the controls (**Figure 4**; **Table 1**).

MDA content of cultivar TH-5 increased significantly upon the foliar application of TU under salinity regime. The two cultivars responded differently for MDA. Cultivar TH-6 showed low MDA content under salt stress and external application of thiourea compared with TH-5 under the same conditions. Salinity imposition significantly elevated SOD activity in cultivar TH-5. Foliar-applied TU did not exhibit a significant impact on SOD activity of both cultivars. The cultivars responded differently for CAT activity. Cultivar TH-6 performed better than cultivar TH-5 in CAT activity. Salinity and TU application had a non-significant impact upon CAT and SOD activities. Salt imposition significantly boosted the POD activity of the sesame plants. Foliar-applied thiourea significantly enhanced the POD activity of the sesame plants. The cultivars differed significantly for POD activity and cultivar TH-6 showed better performance than cultivar TH-6 (**Figure 5**; **Table 1**).

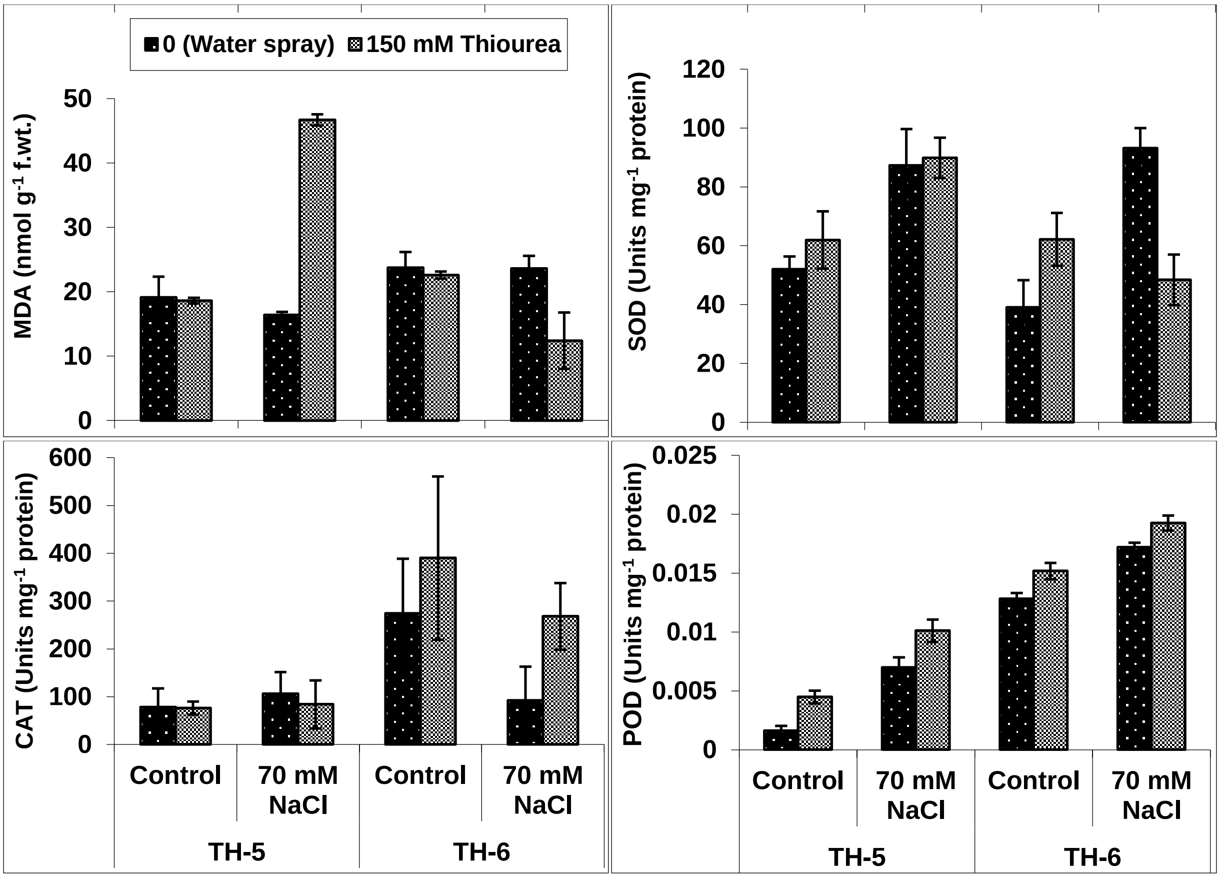

Figure 5. Biochemical parameters of non-stressed and salt-stressed sesame plants treated with foliar-applied thiourea.

Discussion

Salinization of plants is a crucial aspect that causes adverse impacts on growth as well as metabolism to a great extent. Salt stress is known to trigger different mechanisms operating in different organs (Rahneshan et al., 2018). Vegetative growth attributes which include shoot weight (fresh and dry), root weight (fresh and dry), and root and shoot lengths of two sesame cultivars (TH-5 and TH-6) showed considerable reduction due to imposition of salinity and exogenous application of thiourea. Salt-induced suppression in growth has already been observed in different crops such as in maize (Cicek and Cakirlar, 2002), barley (El-Tayeb, 2005), wheat (Anjum et al., 2008) sesame (Gaballah et al., 2007), and sunflower (Sarwar and Shahbaz, 2019). The decline in the growth of plants in the presence of salt stress has been considered to be a consequence of direct reduction of cell division as well as the expansion of the cell (Munns, 2002).

Salinity stress negatively affected chlorophyll *a* content of the sesame cultivars and this diminished impact of salinity was also documented in castor bean (Pinheiro et al., 2008), rice (Amirjani, 2011), tomato (Doganlar et al., 2010), and pigeon pea (Amuthavalli and Sivasankaramoorthy, 2012). Such a reduction in photosynthetic pigments is attributed to gradual synthesis or accelerated disruption of pigments in the cells (Ashraf, 2003). However, foliar-applied TU enhanced the contents of total chlorophyll, chlorophyll *b*, and carotenoids in cv. TH-6 under salt stress. This seems that thiourea palyed an effective role in enhancing the photosynthetic pigments in plants grown under stressful environments.

Sodium (Na^+) increased in the roots and shoots of both sesame cultivars treated with salt stress. Similar findings have earlier been reported in the same plant species (Hota et al., 2016) as well as pearl millet (Hussain et al., 2008). An important component of salt tolerance in the plant is the mechanism of ion homeostasis. An excessive salt application leads to perturbation of this mechanism, besides accretion of toxic ions in various parts of plants (Munns and Tester, 2008). Poor regulation of ion homeostasis results in diminished growth (Kaya et al., 2013). Potassium (K^+) ion content increased in the roots, while decreased in the shoots with the salt application as earlier studied in pearl millet (Hussain et al., 2008) and chaksu (Hussain et al., 2009). Root and shoot Ca^{2+} ion content increased with foliar application of thiourea as already studied in maize (Kaya et al., 2013). Organic compounds like thiourea might play an effectual performance in ionic homeostasis of cells which may result in enhanced uptake of ions like Ca^{2+} (Kaya et al., 2013).

Salinity stress increased the activities of POD and SOD along with the content of malondialdehyde (MDA) in the sesame plants. These results are analogous to what has already been observed in different studies, e.g., enhanced activity of POD in cotton (Meloni et al., 2003) and that of SOD in wheat (El-Bastawisy, 2010) as well as increased levels of MDA in sesame (Gehlot et al., 2005). Development of reactive oxygen species is common in plants under unexceptional and harsh conditions of the environment (Ashraf, 2009). Salt stress also gives rise a boost to the generation of ROS in most plants (Ashraf and Ali, 2008). However, in the present study, foliar application of thiourea declined the activity of antioxidant enzyme catalase (CAT) which might have been due to the antioxidant role of thiourea itself in scavenging the reactive oxygen species (Kaya et al., 2013).

In conclusion, salinity reduced the gas exchange attributes, growth parameters, chlorophyll content and the activity of antioxidant enzyme catalase (CAT), while it enhanced the activity of antioxidant enzymes like POD as well as SOD. Root and shoot Na^+ content increased by the salt exposure. Foliar-applied thiourea raised the POD activity and root K^+ in the presence of salt stress. Overall, it can be inferred that foliar-applied thiourea ameliorates the negative impacts of salinity by enhancing K^+ ion content and antioxidant activity like that of POD.

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Conceptualization and designing the study: MS. Conduction of experiment: SAD. Data collection, visualization and interpretation: NM. Approval of the final version to be submitted: AD.

Ethical approval
This study does not involve Human/animal subjects and no ethical approval is needed.

Handling of bio-hazardous materials
The author(s) certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discorded to minimize any types of bio-contamination(s).

Availability of primary data and martials
As per editorial policy, experimental materials, primary data or software codes are not submitted to the publisher. These are available with corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Author's consent
All authors contributed in designing and execution of the experiment. All contributors have critically read this manuscript and agree for publishing in IJAEB.

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