

## SILICIC ACID AND GLUTATHIONE APPLICATION IMPROVES ELEVATED OZONE TOXICITY TOLERANCE BY MODULATING BIOMASS, SENESCENCE, SEED QUALITY ATTRIBUTES AND OXIDATIVE STRESS INDICATORS IN SESAME (*SESAMUM INDICUM* L.)

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### Abstract

Ever rising ozone (O<sub>3</sub>) is considered as a major threat for crop production in many areas of the world including Pakistan. The present study describes the role of glutathione (0.1 mM) and silicic acid (0.1 mM) for ameliorating the toxic effects of elevated O<sub>3</sub> in Sesame. Four newly released sesame lines, MYT-3, MYT-2, MYT-8, and 1500-I were used in this study as split-split plot design with four replications under ambient (~40 ppb) and elevated (~120 ppb) O<sub>3</sub> treatments. Elevated O<sub>3</sub> toxic effects were recovered through foliar treatment of silicic acid and glutathione via decreased leaf senescence especially at grain filling period, increased chlorophyll pigments (except chl b), increased enzymatic and non-enzymatic antioxidants in addition to decreased MDA and H<sub>2</sub>O<sub>2</sub>. Moreover, the yield attributes were also influenced positively. Negative effects of O<sub>3</sub> on seed oil and protein were reversed with applications but decrease in seed fibre and moisture was not recovered. It is revealed from current findings that silicic acid foliar supplementation is more effective for O<sub>3</sub> toxicity amelioration as compared to glutathione application. Sesame line, MYT-3 produced highest yield under the hazardous O<sub>3</sub> treatment via reducing leaf senescence at grain filling period increasing total chlorophyll, carotenoid contents, SOD and POD, decline in MDA, rise in harvest index and seed oil to prove O<sub>3</sub> tolerant line. This tolerant sesame line could be incorporated in variety development program and subjected to molecular characterization to understand the O<sub>3</sub> stress tolerance mechanism at molecular level in future.

**Key words:** Silicon, GSH, O<sub>3</sub>, Antioxidant, Yield, Seed composition.

### Introduction

The ever changing climate is one of the most important ecological concerns disturbing life on this planet. It is associated with rising of greenhouse gases particularly in developing countries like Pakistan (Hussain *et al.*, 2020; Xu *et al.*, 2021). The elevated tropospheric O<sub>3</sub> exerts noxious effects on biological systems compromising environmental safety and health of living organisms (Nuvolone *et al.*, 2018). It is reckoned as top third pollutant; having greenhouse potential after methane and CO<sub>2</sub> leading to abrupt climate changes including global warming and various lethal effects on biological bodies (Singh *et al.*, 2015; Saleh *et al.*, 2021). It is common observation that less industrialised developing countries have minimum contribution in greenhouse gases emission, but they are at more risk of climate change as compared to developed countries. So, more planning is needed to combat ozone pollution in these regions (Sofia *et al.*, 2020). The adoptive practices may include screening of germplasm, production of O<sub>3</sub> stress tolerant cop varieties, nutrients management under present environmental scenario and exogenous use of plant growth promoting materials (Soares *et al.*, 2019). Detrimental effects of elevated O<sub>3</sub> on plants are reflected through decreased immunity, retarded growth, and low productivity (Sharps *et al.*, 2021). All of these deleterious effects are the outcome of agronomical, anatomical, physiological, morphological, biochemical, and metabolic shifts caused by elevated O<sub>3</sub> concentration in the environment (Leisner and Ainsworth, 2012; De Marco *et al.*, 2021). According to Intergovernmental Panel on

Climate Change (IPCC), O<sub>3</sub> can boost up 70% plant injuries (Sicard *et al.*, 2017). American Environmental Protection Agency (EPA) has reported 10% decline in major crops yield due to O<sub>3</sub> stress in United States, Europe and Asia (Liu and Desai, 2021).

Environmental problems of South Asiatic region are particularly the outcome of the urbanization, increasing population, industrial development, demanding high agricultural productivity and increasing energy consumption (Tariq and Ali, 2015). Pakistan is also facing environmental issues like air pollution. The average ground-level increase in O<sub>3</sub> is 1.04% per year in urban areas of Pakistan since last decade (Zeb *et al.*, 2019). This deleterious molecule is the major constituent of smog (Avnery *et al.*, 2011, Dzierzynska *et al.*, 2012; Usman *et al.*, 2018) which leads to various health issues for many plant and animal species (Ali *et al.*, 2019). Recent considerations of research community are moving toward O<sub>3</sub> stress after drought and salinity, which is reflected by plethora of available literature but in Pakistan, still no particular attention is being provided towards this serious issue of O<sub>3</sub> elevation in atmosphere (Moura *et al.*, 2018).

With extensive industrialization, burning of coal in power plants and vehicle emissions, the O<sub>3</sub> concentration in troposphere is increasing beyond the limits. There are three major sources of O<sub>3</sub> synthesis i.e., coal-fuelled electric plants, transportation (these two contribute 75%), and photochemical industries (Saunier & Blande, 2019).

In Pakistan the prevailing environmental conditions provide favourable circumstances for O<sub>3</sub> synthesis like, sunny days and high mean temperature ranging from 38°C to 48°C.

Sesame is known as the Queen of oil seeds due to high contents (up to 60%) of medically useful edible oil (Mushtaq *et al.*, 2020; Wei *et al.*, 2022). Sesame oil has been declared suitable for health, due to fitness supporting properties (Hsu & Parthasarathy, 2017; Pathak *et al.*, 2017). Though the sesame is an important and beneficial oil seed plant but unfortunately it is not yet listed in O<sub>3</sub> sensitive, moderately sensitive, or resistant species in spite of the fact that its yield is on continuous decline. Foliar nourishing is the exogenous application of plant growth regulators, stimulators, essential nutrients, and a large no of valuable substances which promote plant growth and development even in poor nutritive environment (Laane, 2018).

The O<sub>3</sub> stimulated generation of ROS activates plant defence mechanism which initiates scavenger production like glutathione along with others i.e., non-enzymatic antioxidant and enzymatic antioxidants (Ge *et al.*, 2021). Glutathione has the potential to regulate the biochemical functions of cell (Foyer & Noctor, 2011). Antioxidant capability and peroxidase hunting make glutathione a peroxidase and ROS scavengers along with its role in cell division, growth, and development (Zechmann *et al.*, 2011). Crop resistance toward differential environmental stresses like water scarcity (Nahar *et al.*, 2015), salt stress (Zhou *et al.*, 2016), temperature stress (Zhu *et al.*, 2016), and heavy metal stress (Estrella-Gómez *et al.*, 2012) can be increased through foliar application of glutathione however the O<sub>3</sub> toxicity is yet not investigated.

Silicon, the abundant element in the soil after oxygen and is not listed in the essential element for plants until now (Hussain *et al.*, 2020). It is reported that plants can complete their life span in the absence of silicon but its presence boosts the plant life activities and protection against stresses (Sahebi *et al.*, 2015). Silicon accelerates stress tolerance by inducing rigidity in old leaves resulting on exposure to high sunlight ((Liu & Desai, 2021). Rizwan *et al.*, 2019). Secondly, it increases the light absorption capacity of the plant through surface modification and light transmission character by the deposition of a silica layer on the plant tissue (Seisenbaeva *et al.*, 2021). Different scientific reports vote for the favour of the statement that, reactive oxygen species hunting potential is developed by silicon through oscillating its concentration or function. Yan *et al.*, (2018) reported that SOD and POD activity in the barley crop is increased under the imposition of elevated O<sub>3</sub> concentration. H<sub>2</sub>O<sub>2</sub> is a toxic ROS in plants growing under O<sub>3</sub> stress and is suggested to decrease its accumulation under the influence of exogenously applied silicon in various crops (Abbas *et al.*, 2015; Abdel-Haliem *et al.*, 2017). Vegetative growth and yield data in the field experiment, conducted on many plants displays positive correlation with the exogenous application of silicon compounds which is related to better uptake and translocation of nutrients (Laane, 2018; Pavlovic *et al.*, 2021). Considering the above mentioned effects, it was hypothesized that exogenously applied silicic acid and glutathione may effectively increase growth, oxidative defence and grain yield quality of sesame under O<sub>3</sub> stress. Therefore, the objective of the study was to evaluate the interference of exogenous silicic acid and glutathione

application in leaf senescence, chlorophyll pigments, various enzymatic and non-enzymatic antioxidants, yield and seed composition in sesame under elevated O<sub>3</sub> stress.

## Materials and Methods

**Experimental design and ozone treatment:** The experiment was carried out using a split split-plot experimental design with four replications during the year 2020. Four sesame advance lines MYT-3, MYT-2, MYT-8, and 1500-I were used in the present investigation. Glutathione and silicic acid (Sigma Aldrich, Germany) were applied through foliar application under O<sub>3</sub> stress. Tween-20 was added as a surfactant. The O<sub>3</sub> treatment at the vegetative stage was applied for a consecutive 21 days period. Stress (O<sub>3</sub>) was applied after sunrise till sunset. Two levels of O<sub>3</sub> were used in the experiment i) Ambient (~40 ppb) ii) Elevated (~120 ppb). Each experimental unit was covered by polythene sheath after sunset (like glasshouse) to mitigate the temperature interference till the sunrise. The O<sub>3</sub> stress was applied by O<sub>3</sub> generator (Model No. AOT-MD-500) using oxygen cylinder. For equal O<sub>3</sub> distribution it was applied through a pipe having pore after every 01 ft distance to release it throughout the length of the pipe entering from one side of the elevated O<sub>3</sub> stressed experimental unit till the end. This treatment was consisting of 10 hours repeated after 14 hours for consecutive 21 days. The O<sub>3</sub> concentration was measured continuously through O<sub>3</sub> meter (Model No. 0342e UV O<sub>3</sub> photo analyzer). Laser leveled field was used for pre-sowing irrigation. Seeds of 4 sesame lines were hand drilled @ 05 kg ha<sup>-1</sup> on 12 July 2019. Foliar application of chemicals was applied just before the start of O<sub>3</sub> stress and repeated twice after a week interval. The data collection was carried out after 15 days of treatment.

**Chlorophyll contents:** The chlorophyll contents (*a*, *b*, *a/b* and total chlorophyll) were determined as described by Davis (1979) using healthy and fresh leaves. The leaf samples from each treatment were homogenized in acetone (80%) separately and centrifuged at 12000 rpm for 10 min. Optical density was measured at 663, 645, and 480 nm wavelengths using a double beam spectrophotometer (Hitachi U-1800, Tokyo, Japan). Chl *a*, chl *b*, and total chl contents were determined as below.

$$\text{Chlorophyll } a \text{ (mg/g f. wt)} = \left[ 12.7(\text{OD}_{663}) - (\text{OD}_{645}) \times \frac{V}{1000} w \right]$$

$$\text{Chlorophyll } b \text{ (mg/g f. wt)} = \left[ 22.9(\text{OD}_{645}) - 4.68(\text{OD}_{663}) \times \frac{V}{1000} w \right]$$

$$\text{Total chlorophyll (mg/g f. wt)} = \left[ 20.2(\text{OD}_{645}) + 8.02(\text{OD}_{663}) \times \frac{V}{1000} w \right]$$

Carotenoids contents (mg g<sup>-1</sup>) from fresh weighed leaves were calculated using Kirk and Allen (1965) protocol. Carotenoids = 4.16 (A 480) - [0.89 (A 663) (V /1000 W)]. Where, OD = Optical density, V = Sample volume, W = Sample fresh weight and A = Absorbance

**Enzymatic antioxidants estimation:** Enzymes having antioxidant potential were extracted in 05 mL volume ice-cold phosphate buffer having pH 7.8 from 0.5 g fresh sesame leave grinded in pestle and mortar. Homogenate was filtered followed by centrifugation at 15000 rpm for 20 minutes at 4°C. The supernatant was used to determine enzyme activities.

**Assessment of superoxide dismutase (SOD) activity:** The SOD activity was determined by method of Giannopolitis and Ries (1977) with some modification. This methodology is based on nitroblue tetrazolium (NBT) induced hang-up of photochemical reduction measured at 560 nm wavelength. For the assessment of SOD activity 50 µl of supernatant (enzymatic) dropped into the mixture having 50 µM NBT (ethanol liquefied NBT), 1.3 µM riboflavin, 13 mM methionine, 75 nM EDTA, and 50 mM phosphate buffer (pH 7.8). Mixed solution was set inside the hollow cavity with aluminum-coated walls under the 30 W glowing fluorescent bulb. Reaction initiation took place by switching on the florescent light for 5 minute duration and stopped with turning the button off position. This reaction produces formazane from NBT reduction through the light which was read at the 560 nm wavelength. Whole reacting blend except plant released material was kept under florescent light to treat as control. The illuminated mixtures absorbance was measured at 560 nm wavelength with the help of UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan). Enzyme required for 1/2 inhibition in NBT reduction at 560 nm compared with the control is called as one unit of SOD. *Assessment of Peroxidase (POD) activity.*

The POD performance was calculated using protocol of Chance and Maehly (1955) with slight modification. Oxidation reaction of guaiacol taking place into the reacting blend is responsible for the POD action. To attain a reaction mixture 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H<sub>2</sub>O<sub>2</sub>, and 0.1 mL supernatant from phosphate buffer (7.8 pH) grinding was used to attain 2 cm<sup>3</sup> total volume. Two min time scan was used to read absorbance after every 20s at 470 nm wavelength using UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan) to analyze absorbance fluctuation in reacting solution due to breakdown of guaiacol. POD enzyme action was demonstrated through (µmol of guaiacol broken-down/min) mg<sup>-1</sup> units of protein. Activity of each POD enzyme unit was responsible for 0.01 unit min<sup>-1</sup> absorbance alteration.

### Non enzymatic antioxidants

**Malondialdehyde (MDA):** The O<sub>3</sub> generated peroxidation of cell membrane (oxidative injury) was investigated through the measurement of malondialdehyde concentration into the tissues by following the method of Cakmak and Horst (1991) with slight changes. Trichloroacetic acid (TCA) solution (0.1% w/v) with 3 ml volume was used for the extraction of a 01g leaf sample. Centrifugation was performed for 15 min on 20,000 rpm using SIGMA 2-16KC (centrifuge machine). Three ml quantity of 0.5% concentrated thiobarbituric acid (TBA) was arranged in 20% TCA to be added to 0.5 ml of the

aliquot. Heating temperature (95 °C) for the blend was maintained in water bath for 50 min duration. The heated blend was cooled with the chilled water addition in the water bathtub to stop the reaction. Second time centrifugation (10 min) was performed at 10,000 rpm using SIGMA 2-16KC (centrifuge machine) followed by measuring the eloquent optical density measurement at 532 and 600 nm using UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan). The MDA quantity was estimated through absorbance difference at 600 and 532 nm by following the equation.

$$\text{MDA level (nmol)} = \Delta (A 532 \text{ nm} - A 600 \text{ nm}) / 1.56 \times 105.$$

The absorption coefficient for calculating MDA is 156 mmol<sup>-1</sup>cm<sup>-1</sup>.

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>):** The protocol of Velikova *et al.*, (2000) was used to assess H<sub>2</sub>O<sub>2</sub> concentration. Fully expanded fresh leaves were used for the grinding purpose with pestle and mortar using TCA as a solvent. Half ml of potassium phosphate buffer (pH 7.0) was mixed into the same quantity of leaf extract and one molar potassium iodide (1 ml). Vortexed the solution mixture and read the OD at 390 nm using a UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan). Distilled H<sub>2</sub>O was used as blank.

**Anthocyanin:** The contents were calculated using methodology of Mirecki & Teramura (1984). Healthy and fresh leaves (50 mg) were grinded in 250 µl of acidic (1% HCl, w/v) methanol solution. The grinding of leaf samples was performed on ice followed by 4°C incubation with moderate shaking. Suspensions were removed using a centrifuge machine at room temperature, 14,000 rpm for 05 min. A wavelength of 530 and 560 nm was set on a UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan) to read the absorbance. Anthocyanins calculation was made by following formula:

$$Q \text{ Anthocyanins} = (A_{530} - 0.25 * A_{657}) \times M^{-1}$$

where Q Anthocyanins is a corrected absorption value linearly correlated with the amount of anthocyanin, A<sub>530</sub> and A<sub>657</sub> is the absorption at the indicated wavelengths, and M represents to leaf mass used in extraction (g).

**Total free amino acid:** The total concentration of the free amino acids was assayed using methodology adopted by Hamilton *et al.*, (1943). Field fresh leaves (0.5 g) were grinded with pestle and mortar using phosphate buffer (pH 7.8) as a solvent. One ml quantity of eloquent was mixed with the same volume of pyridine (1%) and ninhydrin (2%). Further, solution was heated up for 30 mints followed by attaining 50 ml final volume with the deionized water to note the optical density setting 570 nm wavelength on UV-visible spectrophotometer (Hitachi U-1800, Tokyo, Japan). Free amino acids were calculated using the following formula:

$$\text{Free amino acids} = \text{Abs} \times V \times DF \div \text{Wt of sample} \times 100$$

**Leaves characteristics at maturity:** Total number of attached leaves was counted when plants attained maximum height. Plants used for total number of leaves count were tagged to estimate leaf senescence. After passing 80, 90 and 100 days from sowing, total number of leaves attached on plant was counted. Difference between total number of leaves and number of leaves after 100 days passed were the dropped leaves.

**Harvest analysis:** Mature crop plants were harvested at the soil surface and immediately weighted. Plants after harvest weight were kept in the electrically powered oven for 72 hours at 70°C to find out the oven dry weight. One hundred healthy seeds were counted and weighted using electric balance. All the healthy seeds of a plant were weighted using electric balance. Ratio of biological yield and seed yield is known as harvest index. The harvest index (%) determination was performed using below mentioned equation:

$$\text{Harvest index \%} = \frac{\text{Seed yield kg ha}^{-1}}{\text{Biological yield kg ha}^{-1}} \times 100$$

**Seed quality characteristics:** Seed oil (%), Seed moisture (%), seed protein and seed fiber were estimated using FT-NIR analyzer by BRUKER OPTIK GMBH, Germany.

**Data analysis:** The collected data were subjected to statistical analysis to test the significance of differences among mean values using CoStat® software version

6.303. Logarithmic transformations were carried out for data normalization, where necessary, prior to analysis. The Student Newman Keuls Test was applied at 5% probability level to analyses the significance of differences among mean value (Steel *et al.*, 1997).

## Results and Discussion

**No. of leaves plant<sup>-1</sup>:** Elevated O<sub>3</sub> concentration significantly ( $p > 0.01$ ) decreased the number of leaves plant<sup>-1</sup> in all studied lines of sesame as stated in Figure 1. Foliar application of glutathione and silicic acid increased the number of leaves plant<sup>-1</sup> in all studied subjects of sesame significantly ( $p > 0.001$ ) but the extent of response in sesame lines and treatment was specific. In case of foliar application of glutathione and silicic acid line 1, 2 and 4 showed an increase in number of leaves plant<sup>-1</sup> under O<sub>3</sub> stress but a decrease in line 3 was observed due to glutathione treatment (Fig. 1). However, under ambient O<sub>3</sub> conditions, an increase in number of leaves plant<sup>-1</sup> was recorded in all of four studied lines when treated with glutathione, silicic acid. Comparatively, silicic acid was found better in increasing number of leaves plant<sup>-1</sup> and line 2 showed highest number of leaves plant<sup>-1</sup>. Previous studies have reported that under O<sub>3</sub> stress the number of leafs per plant is reduced. May be due to accumulation of ROS in apoplast (Fiscus *et al.*, 2005). Significant reduction in plant growth indicated by reduced number of leafs per plant might be due to imbalance of assimilate partitioning among different parts of the plants which ultimately lessens the number of leafs per plants (Ghosh *et al.*, 2020).

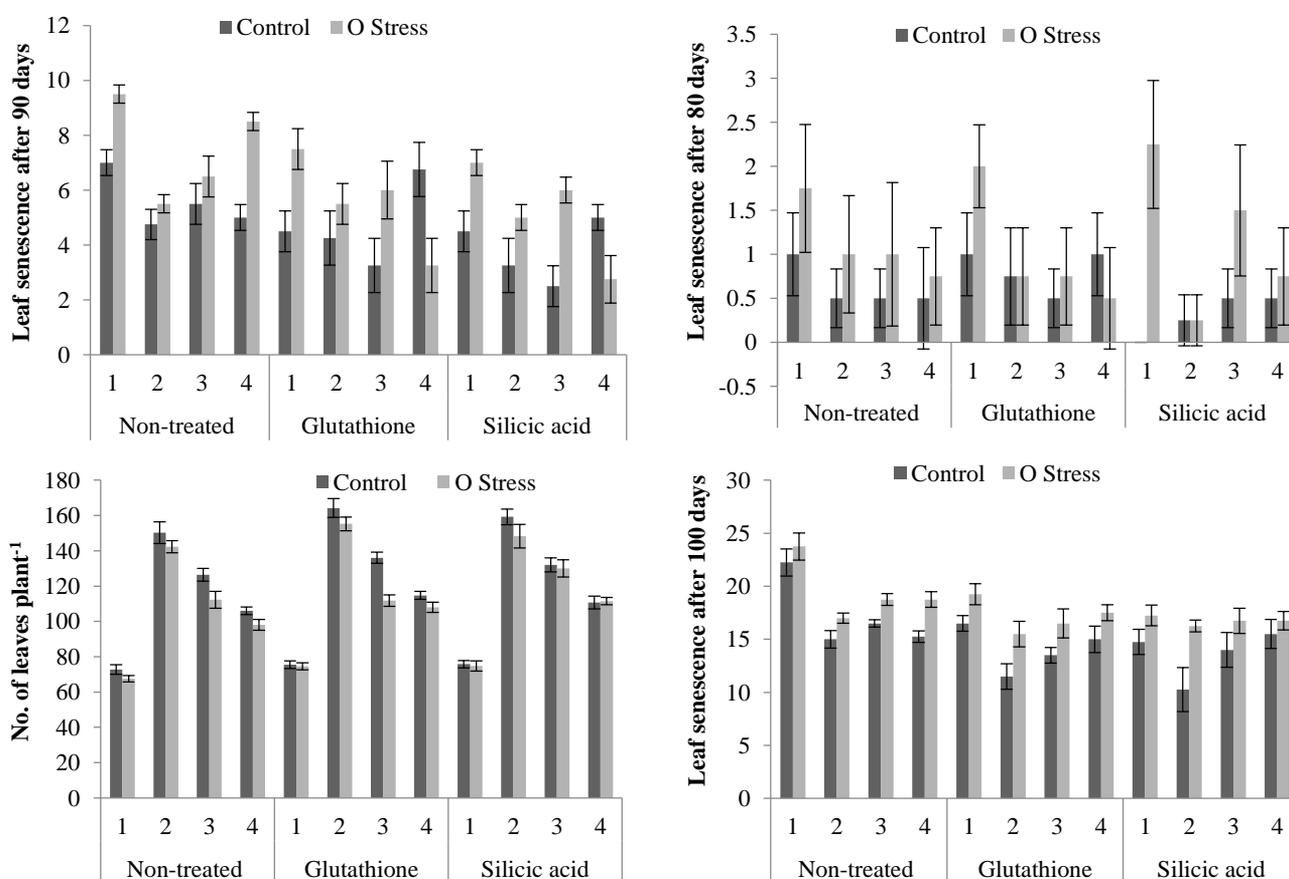


Fig. 1. Effect of glutathione and silicic acid on number of leaf and leaf senescence in sesame lines under elevated level of tropospheric O<sub>3</sub>.

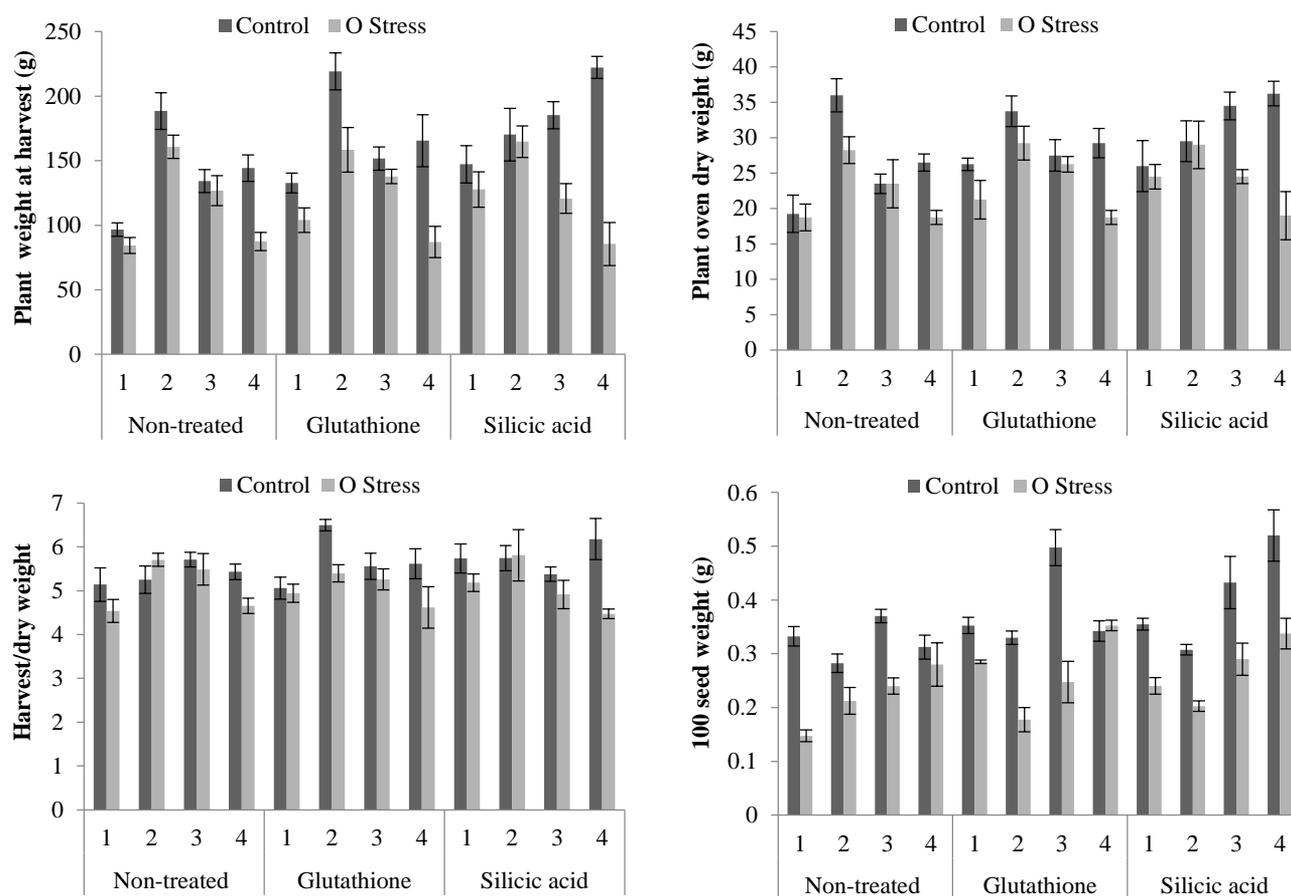


Fig. 2. Effect of glutathione and silicic acid on Plant weight at harvest, oven dry weight and harvest/dry weight in sesame line under elevated level of tropospheric  $O_3$ .

**Senescence attributes:** Elevated level of  $O_3$  resulted in significant increase in leaves senescence after 80 ( $p>0.05$ ), 90 ( $p>0.001$ ) and 100 days ( $p>0.01$ ) as indicated in (Fig. 1). A significant ( $p>0.05$ ) increase in senescence at harvest and significant decrease for attached/senescence was observed (Fig. 1) in all studied lines of sesame. Foliar application of glutathione and silicic acid significantly decreased the senescence leaves after 90 ( $p>0.001$ ), 100 ( $p>0.001$ ) days. The attached/ senescence leaf ratio of all sesame lines was significantly increased but the extent of response in sesame lines and treatment was specific. Comparatively, silicic acid foliar application performed better in decreasing leaf senescence after 80, 90 and 100 days and senescence at harvest (except line 3). The attached/ senescence ratio was increased by foliar application of silicic acid as compared to glutathione application. Among the lines, line 2 showed lowest senescence after 80, 90, 100 days and at harvesting. An increase in attached/senescence ratio was observed in line 2 as well.

Senescence describes unexpected morpho-physiological deviations in leaf flower and fruit (Gan, 2003). It has been documented that plant and leaf life span is reduced under the elevated  $O_3$  level which results in early maturity and senescence of leaf (Black *et al.*, 2007) due to increase in ROS generation in the apoplast (Fiscus *et al.*, 2005). If premature senescence occurs it reduces life span which is reflected into low yield (Hong

*et al.*, 2018). The results of current study also revealed that elevated  $O_3$  increases leaf senescence which is decreased significantly under foliar nebulization of silicic acid and glutathione. All these findings are in line with previous studies showing similar effects under  $O_3$  stress (Ribas *et al.*, 2005; Yendrek *et al.*, 2017; Podda *et al.*, 2019). Current study also uncovered the secret that at early flowering stages (80 days after sowing)  $O_3$  influence on leaf senescence is minor as compared with later stages (90 and 100 days after sowing) because senescence initiation triggered by ripening fruit through the initiation of catabolism (the breakdown of chlorophyll) in the leaf which fulfill high energy demand of fruit at filling and ripening period because only photosynthesis is not enough to supply energy contents for fruit ripening (Buchanan- Wollaston *et al.*, 2002). Lim *et al.*, (2003) also reported the same findings stating that annual plants disassemble reserved nutrients into the leaves and other parts for future investment in the form of next-generation which is in the form of seeds. Elevated  $O_3$  level resulted in significant ( $p>0.01$ ) decrease in attached/senescence ratio for all the studied lines in sesame (Fig. 2). Foliar application of glutathione and silicic acid significantly ( $p>0.001$ ) increased attached/senescence ratio under elevated and normal  $O_3$  concentration. Comparatively silicic acid increases more attached/senescence ratio than glutathione, among lines, line 2 performed best in terms of attached/senescence ratio. Ding *et al.*, (2015) reported that glutathione

application is involved in stress tolerance through delaying in leaf senescence. Like wise our findings particularize that exogenous application of glutathione results into decrease in leaf senescence especially at the grain filling period where O<sub>3</sub> stress is more influential. It is well known fact that silicon, abundant element after oxygen is very useful for plants (Liang *et al.*, 2017). In this study, foliar sprinkling of silicic acid showed more contribution to decrease leaf senescence character especially at most O<sub>3</sub> stress affected stage (grain filling). Alamri *et al.*, (2020) reported that silicon postpones leaf senescence under various stresses in agreement with the results of Markovich *et al.*, (2017).

**Plant weight at harvest, oven dry weight and harvest/dry weight:** The O<sub>3</sub> stress significantly ( $p>0.01$ ) decreased plant weight at harvest stage in all studied four lines of sesame (Fig. 2). Foliar application of glutathione and silicic acid significantly increased ( $p>0.001$ ) plant weight at harvest in all sesame lines but the extent of response in sesame lines and treatment was specific. In case of glutathione line 1 and 3 showed an increase under elevated O<sub>3</sub> concentration but a decrease was observed in line 2 and 4. However, under normal O<sub>3</sub> conditions an increase in plant weight at harvest was recorded in all of four lines. With foliar application of silicic acid under accelerated O<sub>3</sub> concentration a significant increase in plant weight at harvest was found in line 1 and 2. In case of normal O<sub>3</sub> conditions, foliar spray of silicic acid significantly increased the plant weight at harvest in all lines except line 2. Comparatively silicic acid decreases more plant weight at harvest than glutathione among lines, line 2 shows highest plant weight at harvest. Elevated O<sub>3</sub> conditions leads to a marked increment ( $p>0.001$ ) for plant oven dry weight in all of four studied lines in sesame (data represented in fig 2 for plant oven dry weight). Foliar sprinkling of glutathione and silicic acid verified effective strategy to increase ( $p>0.01$ ) plant oven dry weight. In case of glutathione and silicic acid foliar sprinkling line 2 showed small decrease line 1 and 3 increase and line 4 remains unaffected for plant oven dry weight under normal ozone circumstances while all of four lines increases plant oven dry weight under elevated O<sub>3</sub> environment. Comparatively silicic acid increases more plant oven dry weight than glutathione among lines, line 2 shows highest plant oven dry weight.

In present investigation, we found that sesame biomass at harvest time, plant dry mass, and harvest/dry weight ratio were adversely affected by ozone stress. A higher concentration of ozone in the troposphere showed a negative impact on the dry mass production of plants (Li *et al.*, 2016; Han *et al.*, 2020). Plant mass reported to decrease under ozone stress reported by Pei *et al.*, (2019). In current inquiry, ozone stress significantly reduced the seed weight/yield plant<sup>-1</sup>, 100 seed weight and harvest index in sesame crop. Ashrafuzzaman *et al.*, (2017) reported that ozone attacks on plant yield via marked reduction. Ozone attacks on the cereal and oil seed crop's yield characteristics like 100 grain weight (Biswas and Jiang, 2011; Singh *et al.*, 2018) and harvest index. Silicon

is a valuable element for crops; its beneficial effects have been verified by Chen *et al.*, (2018). Present research represents similar results that exogenous application of silicon increased plant harvest weight, plant dry weight, harvest/dry weight ratio, seed weight/yield plant<sup>-1</sup>, 100 seed weight and harvest index. Glutathione is proved a reactive oxygen species scavenger through increasing yield plant<sup>-1</sup> under stressed circumstances while 100 seed weight attribute reflected through increment at lower level (Akram *et al.*, 2017). Plant biomass and yield is also reported to increase under stress full conditions (Pei *et al.*, 2019). In agreement with these reports foliar application of glutathione increased plant harvest weight, plant dry weight, harvest/dry weight ratio, seed weight/yield plant<sup>-1</sup>, 100 seed weight and harvest index.

**Yield variables:** Elevated O<sub>3</sub> level resulted in highly significant decrease ( $p>0.001$ ) in yield plant<sup>-1</sup> and 100 seed weight in addition with marked reduction ( $p>0.01$ ) for harvest index in all studied four lines of sesame (Fig. 3). Glutathione and silicic acid foliar application increased the yield plant<sup>-1</sup> and 100 seed weight of all sesame lines significantly ( $p>0.001$ ) but the extent of response in sesame lines and treatment was specific. Foliar sprinkling of glutathione decreased harvest index while silicic acid resulted in an increase. Comparatively silicic acid increased more yield plant<sup>-1</sup>, 100 seed weight and harvest index than glutathione in all of studied line accept line 1. Among lines, line 1 shows highest yield plant<sup>-1</sup> and line 4 shows highest harvest index and 100 seed weight. In present investigation, we found that sesame biomass at harvest time, plant dry mass, and harvest/dry weight ratio were adversely affected by O<sub>3</sub> stress. A higher concentration of O<sub>3</sub> in the troposphere showed a negative impact on the dry mass production of plants (Han *et al.*, 2020; Li *et al.*, 2016). Plant mass decrease has been reported under O<sub>3</sub> stress by Pei *et al.*, (2019). In current inquiry, O<sub>3</sub> stress significantly reduced the seed weight/yield plant<sup>-1</sup>, 100 seed weight and harvest index in sesame crop. Ashrafuzzaman *et al.* (2017) reported that O<sub>3</sub> attacks on plant yield via marked reduction. Cereal and oil seed crop's yield characteristics like 100 grain weight and harvest index are highly affected under O<sub>3</sub> stress (Biswas and Jiang, 2011; Singh *et al.*, 2018). Silicon is a valuable element for crops; its beneficial effects have been verified by Chen *et al.*, (2018). Present research represents similar results that exogenous application of silicic acid increased plant harvest weight, plant dry weight, harvest/dry weight ratio, seed weight/yield plant<sup>-1</sup>, 100 seed weight and harvest index. Glutathione is proved a ROS scavenger through increasing yield plant<sup>-1</sup> under stressed circumstances while 100 seed weight attribute reflected through increment at lower level (Akram *et al.*, 2017). Plant biomass and yield is reported to decrease under stress full conditions (Pei *et al.*, 2019). In agreement with these reports foliar application of glutathione increased plant harvest weight, plant dry weight, harvest/dry weight ratio, seed weight, yield plant<sup>-1</sup>, 100 seed weight and harvest index in all sesame lines under O<sub>3</sub> stress.

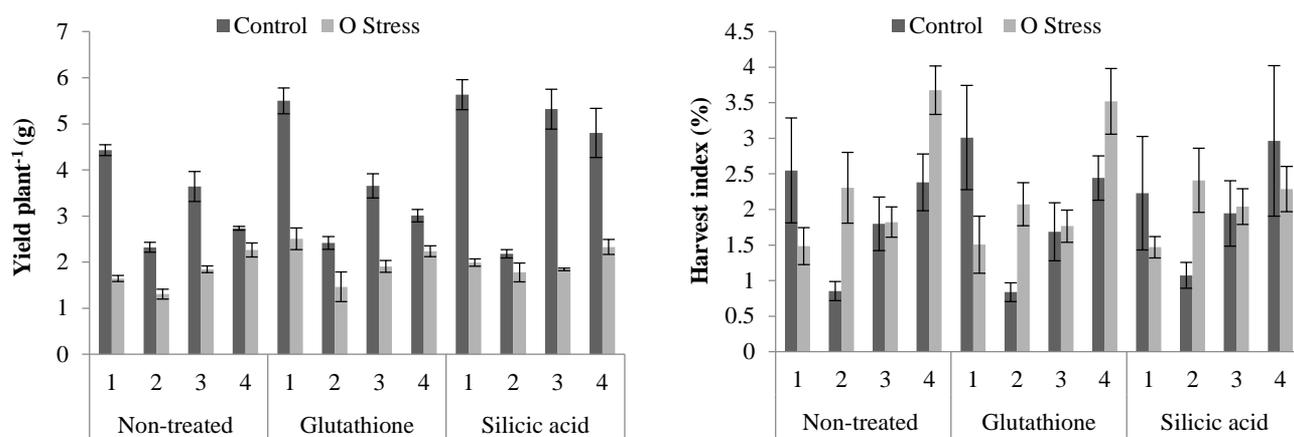


Fig. 3. Effect of glutathione and silicic acid on yield attributes in sesame lines under elevated level of tropospheric O<sub>3</sub>.

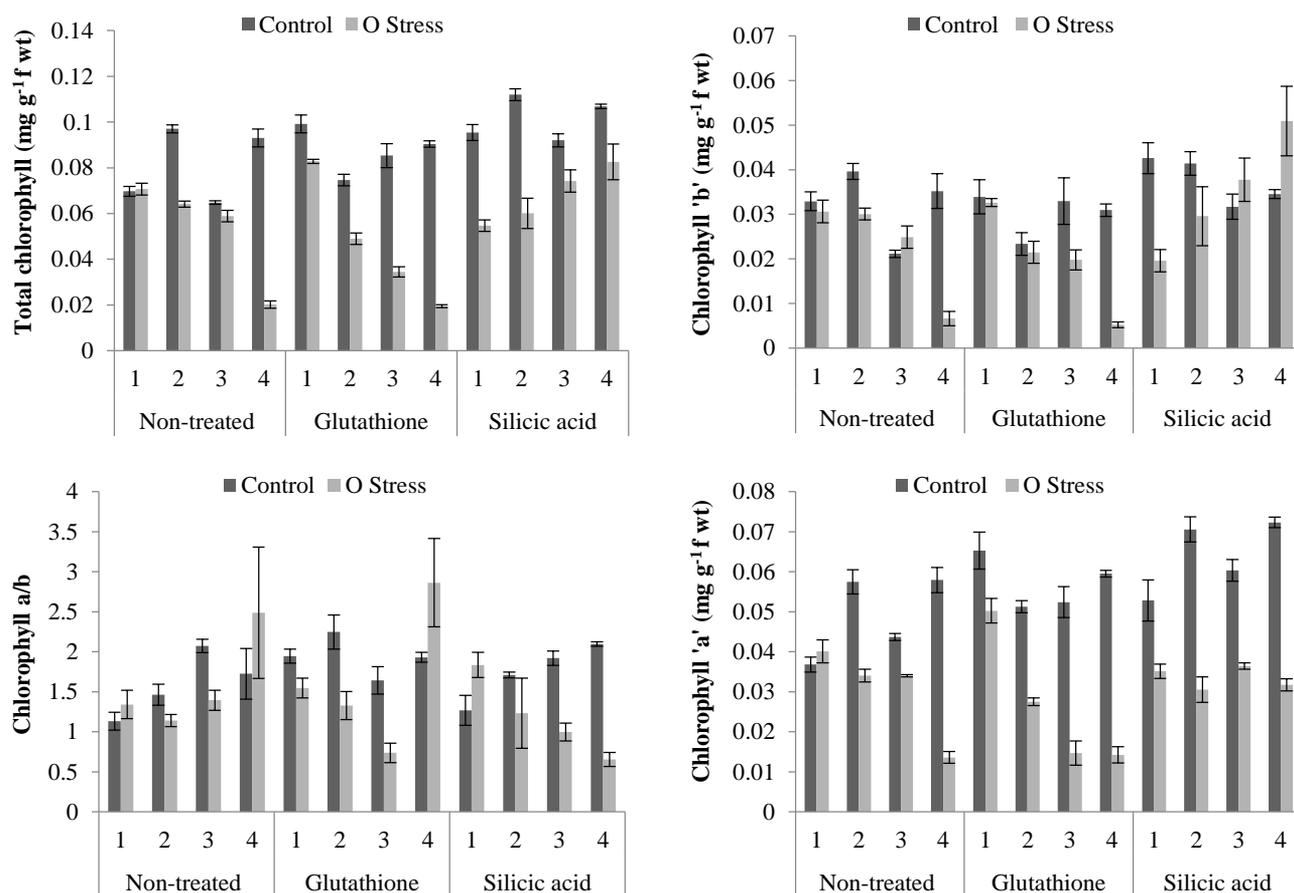


Fig. 4. Effect of glutathione and silicic acid on photosynthetic pigments in sesame lines under elevated level of tropospheric O<sub>3</sub>.

**Chlorophyll contents:** Chlorophyll “a” contents showed significant ( $p>0.001$ ) decrease under enriched O<sub>3</sub> concentration in all the subjects of sesame lines under study. Likewise chlorophyll “b” total chlorophyll and chlorophyll a/b ratio was significantly decreased with slight differences under O<sub>3</sub> stress conditions (Fig. 4). Carotenoid contents in all studied four lines of sesame were also reduced under stress. Foliar spray of glutathione and silicic acid significantly ( $p>0.001$ ) increased the chlorophyll “a” contents of all sesame lines but the extent of response in sesame lines and treatment was specific.

Foliar application of glutathione decreased while silicic acid increased chlorophyll “b” contents and total

chlorophyll contents. Glutathione application increased but silicic acid decreased the chlorophyll a/b ratio and carotenoid contents of all sesame lines but the extent of response in sesame lines and treatment was specific. Comparatively, silicic acid increased chlorophyll “a” contents, chlorophyll “b” contents and total chlorophyll contents bit better as compared to glutathione. Among lines, line 1 showed highest chlorophyll “a” contents, chlorophyll “b” contents, total chlorophyll contents and carotenoid contents and line 4 showed highest chlorophyll a/b ratio. Chlorophyll, the apparatus used in photosynthesis for light absorption is negatively influenced by the exposure to environmental fluctuation or stresses, so it

could be regarded as a sign of crop fitness (Liang *et al.*, 2017). Photosynthetic pigments like chlorophyll and total carotenoid contents located in the leaf are adversely affected by the elevated ozone concentration (Zhang *et al.*, 2016; Han *et al.*, 2020). Similar results were reported by Mahdieh *et al.*, (2018) saying that chl *a*, *b*, and total chlorophyll exhibit a decreasing trend under elevated O<sub>3</sub> concentration. Carotenoid contents in the leaf also decrease under O<sub>3</sub> pollution, as it is reported by Zhang *et al.*, (2016) and Han *et al.*, (2020) which resembles to present investigation. In contrast to present investigation carotenoids increase due to O<sub>3</sub> is also reported in the literature by Pellegrini *et al.*, (2019). Previous investigations show that silicon applied on various crops increases chlorophyll under the imposition of different environmental stresses (Pei *et al.*, 2010; Lu *et al.*, 2018; Wang *et al.*, 2019; Zhang *et al.*, 2018). Foliar application of silicon is effective approach to maintain chloroplast integrity (chl *a*, chl *b* and carotenoids) to maintain photosynthetic efficiency (Rizwan *et al.*, 2019). Further, Gong & Chen (2012) has reported an increase in total chlorophyll contents under similar set of conditions as that of the present piece of work. In the present study foliar application of silicon also support the above mentioned reports regarding chl *a*, chl *b*, total chlorophyll and carotenoid contents except chl *a/b*. A contradictory report is presented by Gao *et al.*, (2011) that silicon application has no influence on leaf chlorophyll. Glutathione foliar application resulted in an increase of leaf chlorophyll contents as reported by Ding *et al.*, (2017) and Nahar *et al.*, (2015). This experiment also showed that exogenous application of glutathione on leaf leads to non-significant increment in all studied chlorophyll attributes (chl *a*, chl *a/b*, total chlorophyll and carotenoids) except chl *b*.

**MDA and H<sub>2</sub>O<sub>2</sub> contents:** Elevated O<sub>3</sub> concentration resulted in significant ( $p>0.05$ ) increase for MDA and H<sub>2</sub>O<sub>2</sub> contents in all of four studied sesame lines (Fig. 5). Foliar application of glutathione and silicic acid increased MDA contents significantly while decreased ( $p>0.001$ ) the H<sub>2</sub>O<sub>2</sub>. In case of glutathione and silicic acid foliar sprinkling as protector resulted into significant decrease showed by all of the studied line in MDA contents under normal and elevated O<sub>3</sub> environment with the exception of glutathione treated line 1 under ozone stressed condition and silicic acid treated line 3 under normal ozone concentration. Comparatively glutathione decreased more MDA contents while silicic acid decreased more H<sub>2</sub>O<sub>2</sub>. Among lines, line 1 show lowest MDA contents and line 4 shows lowest H<sub>2</sub>O<sub>2</sub>. Oscillating ozone concentration in the environment promotes the synthesis of reactive oxygen species i.e. H<sub>2</sub>O<sub>2</sub> (Gao *et al.*, 2005). Elevated ozone enhanced MDA in sesame, suggesting oxidative damage (Han *et al.*, 2015; Nahar *et al.*, 2015; Rizwan *et al.*, 2018). Past reports demonstrated the fact that environmental fluctuations increase MDA concentration and H<sub>2</sub>O<sub>2</sub> contents in the leaf (Ding *et al.*, 2017). Accelerated ozone proportion in the climate reflected to abrupt changes in plant physiology. For example, ozone concentration in the environment has positive correlation with MDA concentration and H<sub>2</sub>O<sub>2</sub> contents (Long *et al.*, 2018). Results from current experiment also in support of early researches that elevated level of ozone increase MDA and H<sub>2</sub>O<sub>2</sub> contents in the leaf.

Differential results presented by Pellegrini *et al.*, (2019) for MDA contents and Du *et al.*, (2018) for H<sub>2</sub>O<sub>2</sub> contents that they remain un-affected under ozone stress, while Podda *et al.* (2019) reported that H<sub>2</sub>O<sub>2</sub> contents decreased due to ozone increment in the environment and elevated ozone decrease MDA contents are reported by Ashrafuzzaman *et al.*, (2017). H<sub>2</sub>O<sub>2</sub> is a poisonous ROS for the plant produced under ozone stress is suggested to decrease its accumulation under the influence of exogenously applied weapon Si in different crops like grapes, wheat, tomato, sorghum, and rice (Soylemezoglu *et al.*, 2009; Abbas *et al.*, 2015; Abdel-Haliem *et al.*, 2017). In agreement with these observations present investigation showed that exogenous application of silicic acid decrease MDA and H<sub>2</sub>O<sub>2</sub> contents under the imposition of ozone stress to protect plants from these devastating chemicals. Malondialdehyde (MDA) response toward silicon application is negatively reported by the Li *et al.*, (2016) and Rizwan *et al.*, (2019). Foliar supply of glutathione resulted into decrease in MDA contents and H<sub>2</sub>O<sub>2</sub> contents under drought stress conditions in mung bean plant but under heat stress contrasting results were reported (Nahar *et al.*, 2015; Akram *et al.*, 2017). In present investigation foliar sprinkling of glutathione also leads to reduction in the MDA and H<sub>2</sub>O<sub>2</sub> contents.

**SOD and POD:** Augmented O<sub>3</sub> conditions leads to a marked increment ( $p>0.05$ ) for SOD while decreased the POD in all of four studied lines in sesame (Fig. 5). Foliar sprinkling of glutathione and silicic acid verified effective strategy to increase ( $p>0.01$ ) SOD. Foliar spray of glutathione increased while silicic acid decreased the POD of all sesame lines but the extent of response in sesame lines and treatment was specific. Comparatively silicic acid increased more SOD while glutathione increased more POD value. Among lines, line 1 shows highest SOD and POD value. Ozone is reactive oxygen species interact with cell to cause oxidative damage. The plant defense system responds to these abnormalities through acceleration in the activities of superoxide dismutase (SOD) and peroxidases (POD). An increase in antioxidant enzyme's performance under the influence of elevated ozone level at the ground surface was also reported by Zhang *et al.*, (2016). Present investigation also reported that imposition of ozone stress leads to increase in SOD and POD values. The SOD values remains un-changed under the influence of ozone stress is reported by Dusart *et al.*, (2019). Different scientific reports vote for the favor of the statement that, reactive oxygen species hunting potential is developed by silicon by oscillating its concentration or function. Liang *et al.*, (2003) reported that SOD and POD activity in the barley crop is increased due to application of silicon. Exogenous application of silicon on cucumber showed increasing trend in the activities of SOD, and POD (Zhu *et al.*, 2004; Rizwan *et al.*, 2019). In agreement with earlier observation this research reported that exogenous application of silicic acid increased SOD and POD values under the imposition of ozone stress. Pei *et al.*, (2019) reported that activities of SOD and POD increased due to glutathione application under stressed condition. In present study, foliar application of glutathione slightly increased SOD values while POD remains unaffected under ozone enriched circumstances.

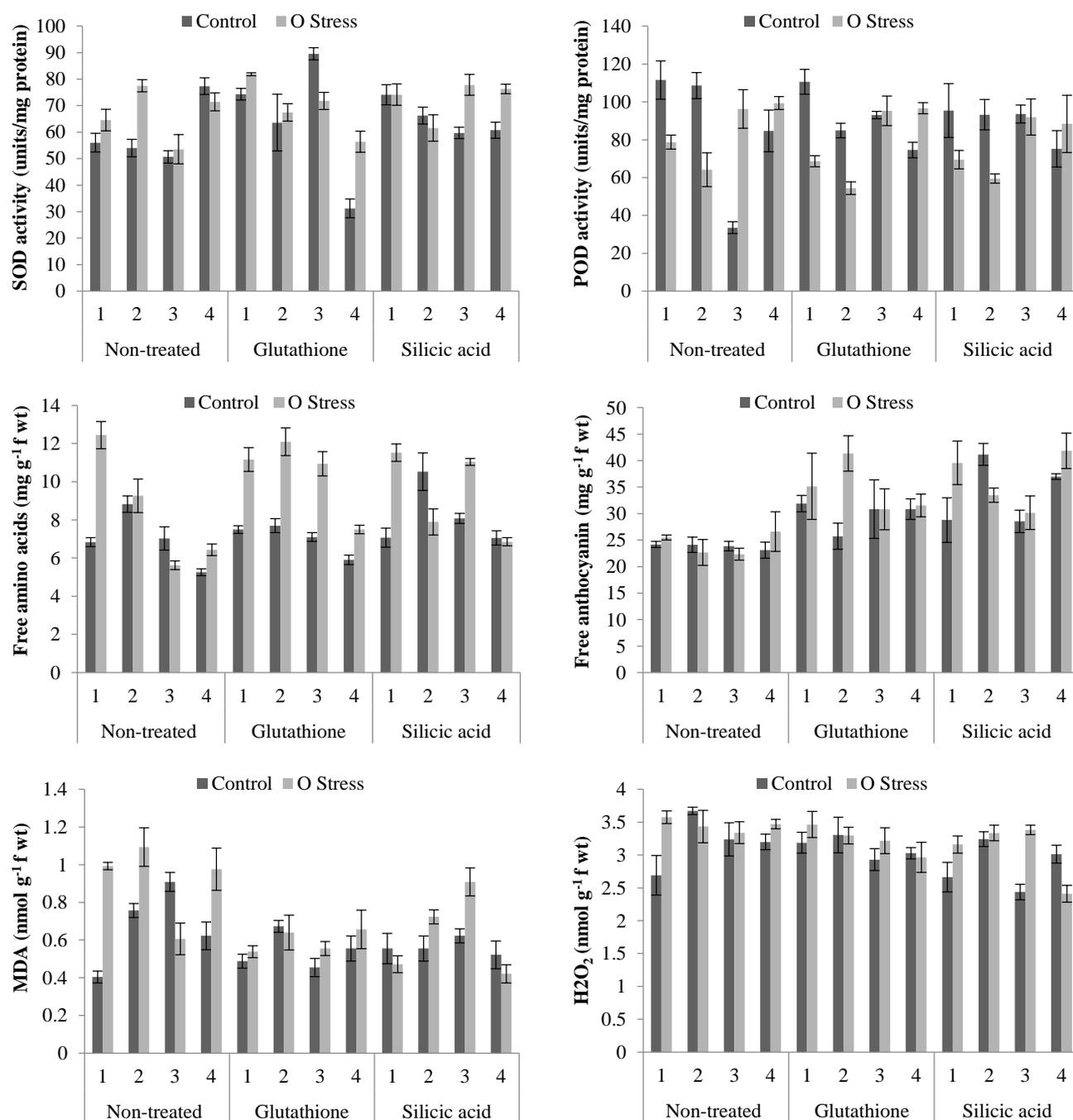


Fig. 5. Effect of glutathione and silicic acid on enzymatic and non enzymatic antioxidants in sesame lines under elevated level of tropospheric O<sub>3</sub>.

**Free-anthocyanin and free amino acids:** Intensified O<sub>3</sub> atmosphere leads to increase ( $p > 0.01$ ) for free-amino acids and free-anthocyanin in all studied four lines of sesame (Fig. 5). Leaves sprayed with glutathione and silicic acid significantly increased ( $p > 0.001$ ) the free-anthocyanin and increase ( $p > 0.001$ ) free-amino acids of all sesame lines but the extent of response in sesame lines and treatment was specific. Comparatively silicic acid increased more free-anthocyanin and free-amino acids than glutathione. Among lines, line 4 shows highest free-anthocyanin and line 1 shows highest free-amino acids. Anthocyanin, the oxidative protector (Pervaiz *et al.*, 2017) increased under the influence of oxidative stresses like elevated ozone level is reported in the past investigations (Chen *et al.*, 2019).

Current analysis performed for the total anthocyanin activity vote in favor of Chen *et al.*, (2019). Metabolic changes in plants depend upon amino acids to a large extent due to the precursor of nucleic acid & proteins. A higher concentration of ozone resulted in diminished amino acid concentration in the leaf extract (Du *et al.*, 2018). In present investigation contradictory results were produced for total amino acid concentration located in the leaf perhaps to create resistance. Silicon is known as a vital and valuable element for plants and it is used for the reversal of harmful effects of a biotic stresses (Gong *et al.*, 2005). Though, silicon play important role to accelerate growth of botanical bodies and demolish adverse effects of stress (Liang *et al.*, 2017). This investigation showed that silicic acid

applications though foliar spray increased both total anthocyanin and free amino acid contents from leaf extract. Hussain *et al.*, (2019) and Kim *et al.*, (2017) also reported that exogenous application of silicon increase antioxidants like free amino acids and anthocyanin. Current investigation reported that exogenous spray of glutathione increase both total anthocyanin and free amino acid contents from leaf extract in agreement with the results of Akladios *et al.*, (2017).

**Seed composition:** Accelerated O<sub>3</sub> concentration in the environment will leads to significant ( $p>0.001$ ) decrease for seed oil contents variable, a marked reduction in seed protein, increase in seed moisture contents, significant ( $p>0.01$ ) increase for seed fiber and in all of four studied lines in sesame (data represented in Fig. 6 for seed oil contents).

Foliar application of glutathione and silicic acid increased seed oil contents, highly significantly ( $p>0.001$ ) increased the seed protein, significantly decreased ( $p>0.001$ ) the seed moisture and seed fiber. Comparatively glutathione increased more seed oil contents, seed moisture contents, seed protein seed fiber than silicic acid. Among lines, line 1 show highest seed oil contents, seed moisture contents, line 4 shows highest seed protein, line 3 shows highest seed fiber. More than half of sesame seed mass is consists of valuable oil followed by proteins and a high proportion of dietary fiber (Brar & Ahuja, 1979; Mushtaq *et al.*, 2020). The sesame oil contents are altered

under the exposure to climate changes. Seed oil contents decreased under the influence of aloft ozone concentration, the severity of the case is linked with the harsh climatic circumstances in context to ozone concentration (Tripathi *et al.*, 2019). Similarly, this investigation also revealed that seed oil contents of sesame significantly decreased while, protein non-significantly decreased under the imposition of ozone pollution. Amount of fatty acid and seed oil contents was also declined in the experiment performed by Singh *et al.*, (2018) under ozone stress. Present analysis showed that moisture in the seed showed almost similar behavior under both conditions. Seed fiber located into the seed showed increasing trend under the influence of elevated ozone in the environment. In contrast to our investigation Sarooei *et al.*, (2019) reported that moisture contents of the seed showed minor reduction under the influence of elevated ozone. Si is famous to alleviate drastic effects of environmental stresses like noxious heavy metal, salinity, drought, chilling and freezing (Liang *et al.*, 2017). Seleiman *et al.*, (2019) demonstrated that silicon improves seed quality like seed oil. In present research work, foliar feeding of silicic acid resulted into increase in the seed oil and protein but seed moisture and fiber decrease. Glutathione, the major water soluble antioxidant in plants increase the seed oil and protein but seed moisture and fiber decrease when applied for foliar nourishment. Awazuhara *et al.*, (2002) reported that seed protein decreased due to glutathione application.

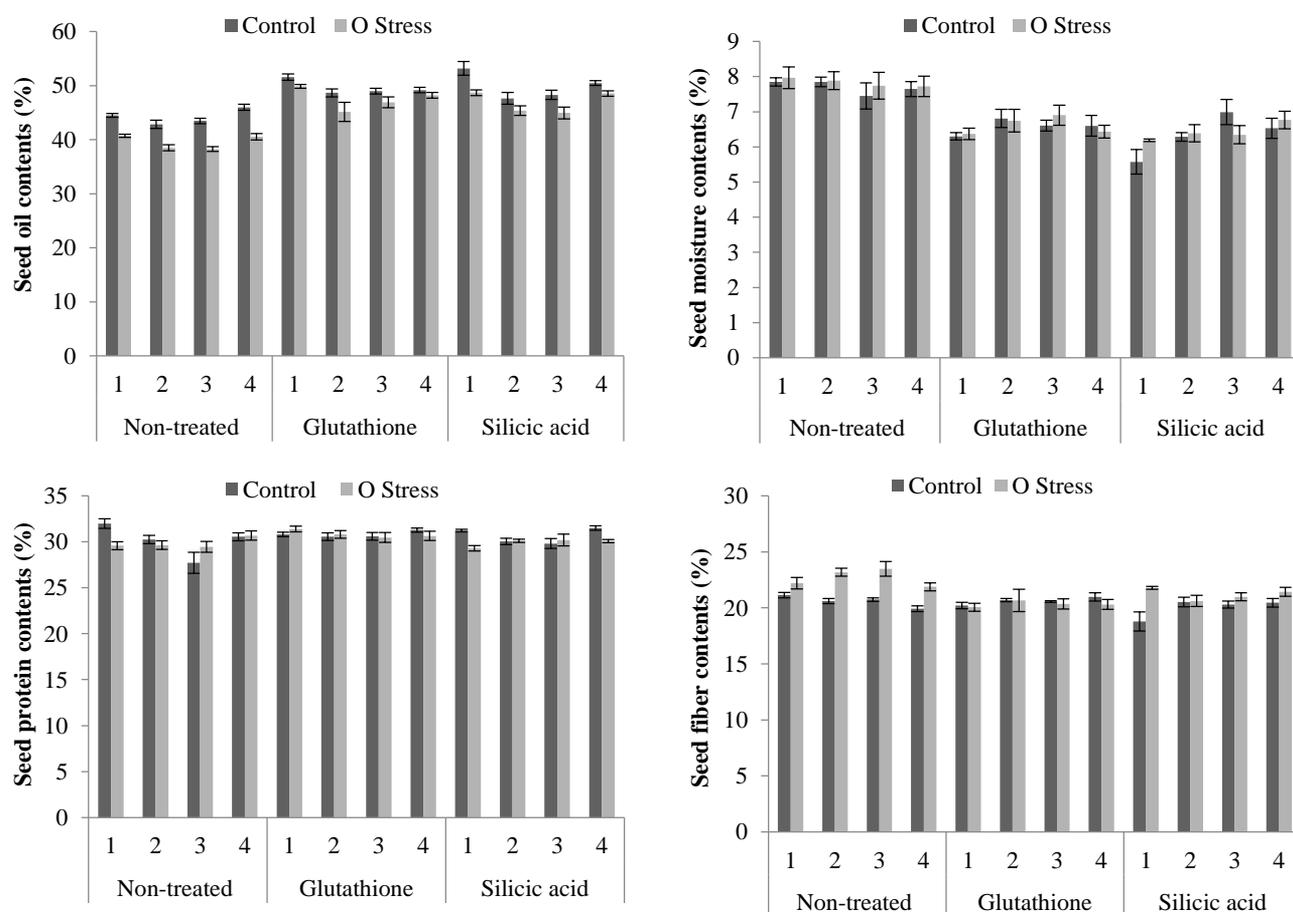


Fig. 6. Effect of glutathione and silicic acid on seed oil, moisture, protein and fiber content in sesame lines under elevated level of tropospheric O<sub>3</sub>.

## Conclusion

Ozone's toxicity for sesame crop is reflected through its detrimental influence on leaf senescence especially at grain filling period, decreased pigments like chl a, chl b, chl a/b, total chlorophyll and carotenoid contents. Enzymatic antioxidants (SOD, POD) and non-enzymatic antioxidants (anthocyanin, free amino acids) are increased. Moreover, yield characters are also ascertained negatively influenced like decreased plant harvest weight, plant dry weight, harvest/dry weight ratio, seed weight/yield plant<sup>-1</sup>, 100 seed weight, harvest index. Foliar nourishment with silicic acid and glutathione decreases leaf senescence and increase chlorophyll pigments. Moreover, regulate biochemical processes like increased anthocyanin and free amino acid and SOD while decreased MDA and H<sub>2</sub>O<sub>2</sub>. Yield attributes increased like plant harvest weight, plant dry weight, yield plant<sup>-1</sup>, 100 seed weight, harvest index, seed oil and protein but decrease in seed moisture and fiber. Sesame line MYT-3 showed maximum yield ignoring the detrimental influence of ozone through decreasing leaf senescence at grain filling period increasing total chlorophyll and carotenoid contents, SOD, POD, reduction in MDA, increment in harvest index and seed oil to prove ozone tolerant sesame line. These results offer effective and useful method to eliminate ozone toxicity that suggests silicon and glutathione have ozone detoxification potential for sesame crop. In future, sesame line MYT-3 could be used as parental blood in variety development program and use of silicic acid may be exploited for biotic and abiotic stress tolerance in this crop.

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