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The Relationship Between Sulphur Dioxide and Trehalose and Their Effect on Some Biochemical Characteristics of Tomato Plants

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Abstract: Sulphur oxide is one of the most serious problems of air pollution on the environment, especially human and plant health. This study was conducted to clarify the effect of sulphur dioxide (SO_2) stress on some of the biochemical characteristics of tomato plant, *Lycopersicum esculentum* Mill. The plants were exposed to three doses of SO_2 (0, 5, 10 $mg.m^{-3}$), the exposure period was inclusive to 1 and 2 hours for each day. In order to reduce the toxicity of sulphur dioxide, the plants were treated by spraying them with trehalose sugar at concentrations of 0,50 and 100 $mg.L^{-1}$. The results of the experiment showed that increasing the concentration of SO_2 as well as increasing its exposure led to an increase in the activity of enzyme antioxidants (superoxide dismutase, SOD; peroxidase, POD; catalase CAT) and non-enzymatic antioxidants (Proline, Vitamin C, Lycopene), while the antioxidant activities decreased when spraying trehalose, especially at concentration 100 $mg.L^{-1}$. It also recorded a positive effect in mitigating the negative effects of sulphur dioxide stress.

Key words: Sulphur dioxide (SO_2), trehalose sugar, superoxide dismutase (SOD), peroxidase POD, catalase (CAT), proline, vitamin C, lycopene.

Introduction

Society today is facing the consequences of air pollution, especially in cities, because pollutants have spread widely everywhere. The most important pollutants are gases that cause harm to human health as well as the environment (nitrogen oxides - sulphur oxides - hydrocarbons - ozone) (Ogunrotim et al., 2017; Uaboi-Egbenni, 2009). Sulphur dioxide is a major air pollutant and abiotic stress factor resulting from fuel consumption due to vehicle smoke, manufacturing and fossil fuels, where it increased by 50% in industrialised countries compared to the last decade (Krotkov et al., 2016). Sulphur dioxide gas is poisonous to living creatures and has an unpleasant and strong odour, but it is a nutrient used by plants to synthesise certain amino acids (Capaldi et al., 2015). Long-term exposure to sulphur dioxide gas has a negative impact on plant growth, as the length of buds reduces after chronic exposure to the gas, and the rate of length and stem diameter falls when exposed to a sulphur dioxide gas concentration more than the permissible limit in the air (Choi et al., 2014; Sharma et al., 2014). Some studies have confirmed that there is a change in the phenotypic characteristics, such as a decrease in the frequency of stomata openings, as well as the distribution of epidermal cells in some plants as a reaction to gases resulting from cars containing sulfur dioxide gas when compared to plants exposed to pollution without sulphur dioxide gas, where the size of the stomata and epidermal cells increased (Vrema

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et al., 2014; Haworth et al., 2012). The main effect of toxic sulphur dioxide gas is due to the production of sulphur (SO_3^{-2}) and bisulphite (HSO_3^-) radicals. After it is dissolved in the cellular water, the plant begins to remove toxins, as the toxic sulphite turns into sulphate radicals, which are less harmful and a by-product of the reactive oxygen species ROS, especially superoxide radicals and hydrogen peroxide (Li and Yi, 2012). Excessive accumulation of ROS is considered harmful and hinders plant functions, as oxidative stress negatively affects proteins and nucleic acids, but the greatest impact is on cellular and membrane lipids, where they are oxidized more quickly by (ROS) (Apel et al., 2005; Foyer et al., 2005).

Trehalose is a non-reducing disaccharide that consists of two glucose molecules linked by an α bond. The main function of trehalose is the regulation of osmosis and the removal of free radicals, as well as the integrity of the protein membrane under various stress conditions (Elbein, 2003). A variety of enzymatic and non-enzymatic antioxidants are produced (Yadav et al., 2014). Trehalose plays an important role in neutralising reactive oxygen species as well as maintaining the protein's anabolic mechanism (Chang et al., 2014). When one stoichiometric molecule of trehalose reacts with a double bond of type (Cis-olefin) of unsaturated fatty acid, a stable compound is formed, which leads to a significant reduction in the oxidation process (Nery et al., 2008). In a study, treatment with trehalose led to a significant increase in soluble sugars (Abdallah et al., 2016). Treating wheat seedlings with trehalose stimulated the catalase activity under stress (Dolatabadian and Jouneghani, 2009). The aim of conducting this experiment is to find out the dose of sulphur dioxide that is most harmful to plant growth and its relationship to enzymatic and non-enzymatic antioxidants of the tomato plant and to reduce this damage by using trehalose sugar.

Materials and Methods

Tomato, *Lycopersicum esculentum*, plants were planted in a 5 kg pot of sandy loam soil pots during the 2020-2021 growing season. The plants were subjected to different SO_2 doses throught an artificial fumigation system. SO_2 was generated by burning the incense burner in fumigation chambers (1 m^3). The experiment was designed as a factorial experiment using a randomise complete block design (RCBD) with three factors and three replicates. The first factor represents three doses of sulphur dioxide (SO_2) at concentrations of 0, 5 and 10 $mg.m^{-3}$. The second factor represents three concentrations of trehalose sugar (T), which are 0, 50 and 100 mg. L^{-1}. The third factor is the gas exposure period (H) (1 hour and 2 hours). Therefore, the total number of experimental units was 54 units. After eight weeks of cultivation, the vegetative part for each treated plant was placed under cooling conditions and extracted to measure the enzymes.

Extraction of Enzymes

About 1 gram of the soft part of the plant was weighed and cut with clean scissors into small parts and soaked in (10) ml of potassium phosphate (K_2HPO_4) solution, then mashed completely, then filtered with a piece of gauze and placed in a cooled centrifuge up to 4 °C at 4000 rpm for half an hour, the filter was isolated in clean tubes and the samples were kept in refrigerated conditions. These samples were used to estimate the activity of the antioxidant enzymes, also studied by Pitotti et al. (1995). The determination of superoxide dismutase (SOD) was based on the method given by Beyer and Fridovich (1987). The peroxidase activity (POD) was determined according to the method of Nezih (1985). Also, the activity of catalase (CAT) was estimated by the Aebi method (1974).

Determination of Proline Content (µg. g^{-1})

About 0.5 g of the vegetative parts was mashed in a ceramic slurry with 3% sulphosalicylic acid at a rate of 10 ml. The samples were filtered using Whatman No (2) filter paper and placed in test tubes. Then 2 ml of ninhydrin solution was placed in a water bath set at a temperature of 100ºC for an hour. The samples were cooled and 4 ml of Telion was added to them and mixed by Fortex, the samples were left for a while until they reached a temperature equal to room temperature, then they were pulled from the upper layer to read the absorbance at 520 nm, the colours of the plants were determined only by a wooden board. The proline concentration of the plants was measured by the relationship between the proline concentration and absorbance as suggested in the study by Bates et al. (1993).

Determination of Ascorbate Content (µg. g^{-1})

Mash 1 gram of the light green part tissues using a ceramic mortar and add to it 10 ml of 0.05 M oxalic acid, then the samples were placed in dark conditions and under cooling conditions for 12 hours or a whole day. The samples were filtered and 2.5 ml of samples were taken. To this 2.5 ml of oxalic was added with

1 ml of H_2SO_4 (5%) and 2.5 acetic-phosphoric acid and added to the filtrate after the addition of 2 ml of ammonium molybdate (5%). The volume of each tube was made up to 25 ml of distilled water, and then the reading of the samples were taken using a Spectrophotometer at 760 nm, and then ascorbate concentrations were calculated using the relationship between the standard concentration of ascorbate and the absorbance values (Hussain et al., 2010).

Determination of Lycopene Content (µg. g^{-1})

The lycopene content was estimated by the Pakutharvu Method (2011). About 5 grams of tissue from the soft vegetative part was preserved in high cold conditions. The tissues samples were cut into small pieces using scissors Then it was mashed in a ceramic slurry with acetone (10 ml), filtered through a piece of gauze, the filtrate was transferred to tubes and 10 ml beryllium ether was added to it. Sodium suphfate concentrates at 5% was added while shaking, the samples was left in the shaker to mix properly for a quarter of an hour. Afterwards, 10 ml beryllium ether was added to make the solution homogeneous and colourless. The absorption was then measured by using a spectrophotometer at a wavelength of 503 nm. The following equation was used to calculate lycopene concentration:

Lycopene concentration = Sample ÷ 13.21 × A_{503}

A_{503}: represents the absorbance reading of the device.

Sample: represents the weight of the sample and is equal to 5 grams.

13.21: represents the unit absorption in centimeters

Statistical Analysis

One-way analysis of variance (ANOVA) were carried out using System (2012)-SAS statistical. Data obtained were analysed statistically to determine using least significant difference (LSD) at $p \leq 0.05$.

Table 1: Effect of timing and addition of sulfur dioxide SO_2 and trehalose on the activity of superoxide dismutase (SOD) units.mg.protein^{-1}

Hour H	*Trehalose mg.L^{-1}*	*SO$_2$ mg.m^{-3}*			*Mean trehalose × H*
		0	*5*	*10*	
1	0	22.52	31.62	38.95	31.03
	50	18.40	35.26	35.49	29.71
	100	17.36	24.14	34.33	25.27
2	0	24.04	38.86	37.49	33.46
	50	20.11	38.52	39.06	32.56
	100	17.57	36.60	38.10	30.75
LSD. 0.05		9.106*			7.91*
SO$_2$ mg.m^{-3} × H					
H		*SO$_2$ mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		19.42	30.34	36.25	28.67
2		20.57	37.99	38.21	32.25
LSD. 0.05		7.91*			4.52NS
SO$_2$ mg.m^{-3} × trehalose mg.L^{-1}					
Trehalose mg.L^{-1}		*SO$_2$ mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		23.28	35.24	38.22	32.24
50		19.25	36.89	37.27	31.13
100		17.46	30.37	36.21	28.01
LSD. 0.05					5.59 NS
Mean SO_2		19.99	34.16	36.87	
LSD 0.05		5.59*			

Results

The results given in Table 1 showed that there was a significant increase in the activity of the enzyme superoxide dismutase (SOD) when sulphur dioxide gas SO_2 was increased (0-10 mg.m^{-3}), as the value increased from (19.99) units.mg.protein^{-1} to (36.87) units. mg.protein^{-1} and, respectively, while the results showed a decrease in the activity of the enzyme Superoxide dismutase with an increase in the concentration of trehalose sugar (0-100) mg.L^{-1}. There was a decrease in the activity of the enzyme superoxide dismutase from 32.24 to 28.01 units. mg.protein^{-1}, respectively, with an increase of 13.12%, and there was a significant decrease in the enzyme superoxide dismutase at the time of exposure 1 hour, reaching 28.67 units. mg.protein^{-1}, compared with the highest value (32.25) units.mg.protein^{-1} at a period of (2) hours, with a rate of 12.48%. The triple interaction between 100 mg.L^{-1} of trehalose and zero concentration of sulphur dioxide gas, while keeping the gas chambers closed for 1 hour, led to a decrease in the activity of the enzyme (SOD) (39.06-17.36) units.mg.protein^{-1}.

The results of Table 2 show that there was a significant increase in the activity of the peroxidase enzyme (POD) when gas SO_2 was increased (0 to 10 mg.m^{-3}) as the value increased from 20.13 unit. mg.protein^{-1} to 49.86 unit.mg.protein^{-1}, respectively, while the results showed a decrease in the activity of the enzyme (POD) with an increase in the concentration of trehalose sugar (0 to 100) mg.L^{-1}, there was a decrease in the activity of the plant enzyme (POD) from 41.69 to 32.90 unit.mg.protein^{-1}, respectively, with an increase of 21.08%, and there was a significant decrease in (POD) enzyme at exposure time: 1 hour, reaching 34.36 unit. mg.protein^{-1} compared with the highest value of 40.30 units.mg.protein^{-1}. The rate of 14.73% of at a period of 2 hours was 14.73%. The triple interaction between 100 mg.L^{-1} of trehalose and zero concentration of sulphur dioxide gas while keeping the gas chambers closed for

Table 2: Effect of timing and addition of sulfur dioxide and trehalose on the activity the peroxidase enzyme (POD) unit.mg.protein^{-1}

Hour H	*Trehalose mg.L^{-1}*	*SO_2 mg.m^{-3}*			*Mean trehalose × H*
		0	*5*	*10*	
1	0	21.89	43.76	52.31	39.32
	50	20.55	36.11	47.86	34.84
	100	13.19	32.58	41.05	28.94
2	0	23.83	51.26	57.13	44.07
	50	21.46	45.77	52.72	39.98
	100	19.91	42.56	48.14	36.87
LSD. 0.05		14.763*			12.17*
		SO_2 mg.m^{-3} × H			
H		*SO_2 mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		18.54	37.48	47.07	34.36
2		21.73	46.53	52.66	40.30
LSD. 0.05		12.17 *			7.016 NS
		SO_2 mg.m^{-3} × trehalose mg.L^{-1}			
Trehalose mg.L^{-1}		*SO_2 mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		22.86	47.51	54.72	41.69
50		21.00	54.44	50.29	41.91
100		16.55	37.57	44.59	32.90
LSD. 0.05		12.87*			8.60*
Mean SO_2		20.13	46.50	49.86	
LSD 0.05		8.60*			

1 hour led to a decrease in the activity of the enzyme (POD) 57.13 - 13.19 units.mg.protein^{-1}.

The results given in Table 3 showed a significant increase in the activity of the enzyme catalase (CAT) when sulphur dioxide was increased (0-10 mg.m^{-3}) as the value increased from 19.98 unit.mg.protein^{-1} to 43.14 unit.mg.protein^{-1}, respectively. The results showed a decrease in the activity of enzyme (CAT) (i.e., 39.41 to 28.97 unit.mg.protein^{-1}) with an increase in the concentration of trehalose from 0 to 100 mg.L^{-1}. However, there was an increase of 26.49% with a significant decrease in CAT enzyme (32.15 unit.mg.protein^{-1}) when the reaction was performed for 1 hour as compared to 37.21 unit.mg.protein^{-1} (15.73%), which was the highest value obtained by performing the reaction for 2 hours. The triple interaction between 100 mg.L^{-1} of trehalose and 0 concentration of sulphur dioxide while keeping the gas chambers closed for 1 hour led to a decrease in the activity of the enzyme (CAT) (51.72-13.24) unit.mg.protein^{-1}. The results of Table 4 showed a significant increase in lycopene concentration when increasing sulphur dioxide gas SO_2 (0-10 mg.m^{-3}) as the value increased from 1.07 µg.gm^{-1} to 1.42 µg.gm^{-1}, respectively, while the results showed decrease in lycopene concentration upon increase in trehalose concentration (0-100) mg.L^{-1}. There was a decrease in plant lycopene concentration from 1.28 to 1.25 µg.gm^{-1} with an increase of 2.34% and a significant decrease in lycopene concentration was achieved for a period of time. Exposure for 1 hour resulted in a yield of 1.20 µg.gm^{-1} compared to the highest value of 1.32 µg.gm^{-1} which was obtained after exposure to 2 hours and was approx. 10.00% more. The triple interaction resulting between 100 mg.L^{-1} of trehalose with sulphur dioxide in a gas chamber, which was kept closed for 1 hour, showed a decrease in lycopene concentration from 1.62 to 0.99 µg.gm^{-1}. Table 5 indicated that there was a significant increase in the concentration of

Table 3: Effect of timing and addition of sulphur dioxide SO_2 and trehalose on the activity of the catalase (CAT) unit.mg.protein^{-1}

Hour H	*Trehalose mg.L^{-1}*	*SO_2 mg.m^{-3}*			*Mean trehalose × H*
		0	*5*	*10*	
1	0	23.45	42.24	45.32	37.00
	50	20.60	38.03	43.63	34.08
	100	13.24	31.25	31.65	25.38
2	0	25.16	48.58	51.72	41.82
	50	21.37	44.16	46.22	37.25
	100	16.13	41.28	40.32	32.57
LSD. 0.05		12.421*			10.86*
SO_2 mg.m^{-3} × H					
H		*SO_2 mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		19.09	37.17	40.20	32.15
2		20.88	44.67	46.08	37.21
LSD 0.05		10.86 *			6.26 NS
SO_2 mg.m^{-3} × trehalose mg.L^{-1}					
Trehalose mg.L^{-1}		*SO_2 mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		24.30	45.41	48.52	39.41
50		20.98	41.09	44.92	35.66
100		14.68	36.26	35.98	28.97
LSD 0.05		11.094*			7.653*
Mean SO_2		19.98	40.92	43.14	
LSD 0.05		7.653*			

Table 4: Effect of timing and addition of sulphur dioxide SO_2 and trehalose on concentration of lycopene content (µg.g^{-1})

Hour H	*Trehalose mg.L^{-1}*	*SO_2 mg.m^{-3}*			*Mean trehalose ×H*
		0	*5*	*10*	
1	0	1.02	1.28	1.34	1.21
	50	1.10	1.22	1.32	1.21
	100	1.12	1.16	1.29	1.19
2	0	0.99	1.44	1.62	1.35
	50	1.11	1.25	1.53	1.30
	100	1.14	1.37	1.44	1.32
LSD. 0.05		0.279 *			0.218 NS
		SO_2 mg.m^{-3} × H			
H		*SO_2 mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		1.08	1.22	1.31	1.20
2		1.08	1.35	1.53	1.32
LSD. 0.05		0.218 *			0.125 NS
		SO_2 mg.m^{-3}× Trehalose mg.L^{-1}			
trehalose mg.L^{-1}		*SO_2 mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		1.00	1.36	1.48	1.28
50		1.10	1.23	1.42	1.25
100		1.13	1.26	1.36	1.25
LSD. 0.05		0.226 *			0.155 NS
Mean SO_2		1.07	1.28	1.42	
LSD. 0.05		0.155 *			

proline when sulphur dioxide gas was increased (0 -10 mg.m^{-3}) as the value increased from 28.42 µg.gm^{-1} to 38.94 µg.gm^{-1}, respectively, while the results showed a decrease in the concentration of proline. With an increase in the concentration of trehalose at (0-100) mg.L^{-1}, there was a decrease in the concentration of plant proline from (38.00-32.27) µg.gm^{-1}. The exposure (1) hour amounted to (33.13) µg.gm^{-1}compared with the highest value (36.13) µg.gm^{-1} over a period of (2) hours, which was 9.09%. The triple interaction resulted between 100 mg.L^{-1} of trehalose and with a zero concentration of sulfur dioxide gas while keeping the gas chambers closed for one hour a decrease in the concentration of proline (43.20 - 25.74) µg.gm^{-1}. The results of Table 6 indicated that there was a significant increase in the concentration of ascorbite when sulphur dioxide gas was increased (0-10 mg.m^{-3}), as the value increased from (13.70) µg.g^{-1} to (16.25) µg.g.$^{-1}$, and there was a decrease in ascorbite concentration. By increasing the concentration of trehalose (0-100) mg.L^{-1}, there was a decrease in the plant ascorbate concentration from (16.34 to 14.22) µg.g^{-1}, which was an increase of 14.90%. There was a significant decrease in concentration of ascorbite at the time of exposure. (1) hour when it reached (14.87) µg.g-^{1} compared with the highest value (15.45) µg.g^{-1} during a 2-hour period at a rate of 9.09%. The triple interaction occurred between 50 mg.L^{-1} of trehalose with a concentration of 0 sulfur dioxide gas while keeping the gas chambers closed for a period of (2) hours decrease in ascorbite concentration (18.50-12.15) µg.g^{-1}.

Discussion

When tomato plants were exposed in gas chambers to sulphur dioxide stress, this led to an increase in the effectiveness of antioxidant enzymes (SOD, POD, CAT) as shown in Tables 1-3. The increase in gas concentration and duration of exposure led to an increase in the non-enzymatic antioxidants (lycopene,

Table 5: Effect of timing and addition of sulphur dioxide SO_2 and trehalose on concentration of proline content (µg.g^{-1})

Hour H	*Trehalose mg.L^{-1}*	*SO_2 mg.m^{-3}*			*Mean trehalose × H*
		0	*5*	*10*	
1	0	31.82	38.02	42.43	37.42
	50	27.20	32.83	35.15	31.72
	100	27.20	30.79	32.75	30.24
2	0	32.12	40.45	43.20	38.58
	50	26.46	39.74	40.38	35.52
	100	25.74	37.40	39.80	34.31
LSD. 0.05		11.107*			8.542 NS
SO_2 mg.m^{-3} × H					
H		*SO_2 mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		28.74	33.88	36.77	33.13
2		28.10	39.19	41.12	36.13
LSD. 0.05		8.496*			3.668 NS
SO_2 mg.m^{-3} × trehalose mg.L^{-1}					
Trehalose mg.L^{-1}		*SO_2 mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		31.97	39.23	42.81	38.00
50		26.83	26.28	37.76	33.62
100		26.47	34.09	36.27	32.27
LSD. 0.05		9.42*			4.514*
Mean SO_2		28.42	36.53	38.94	
LSD. 0.05		4.514*			

proline and ascorbite) as shown in Tables 4-6. The increase in the concentration of sulphur dioxide causes damage to the cell membrane indirectly through an increase in the leakage of ions, as well as an increase in the accumulation of ROS such as H_2O_2 and O_2^- and this leads to an imbalance in the oxidation of plants. Plants contain defense mechanisms that remove oxidising compounds, and when sulphur dioxide gas is stressed, these antioxidant activities increase (Lee, et al., 2017). The excessive accumulation of ROS is considered harmful and leads to problems in the function of the plant in several ways. Oxidative stress negatively affects DNA and proteins, but the most harmful effect is on fats because they oxidise quickly (Foyer & Noctor, 2005; Yi et al., 2005). The increase in the production rates of $H_2O_2^-$ and O_2^- causes an increase in the production of SOD (Huang et al., 2015). The effectiveness of POD and CAT increased significantly for plants exposed to sulphur dioxide gas, where CAT played an important role in reducing ROS while POD plays a specific role in removing sulphites, which results from the dissolution of sulphur dioxide in cellular water (Verma et al., 2014). There are many studies confirming the increase in the effectiveness of antioxidant enzymes when the plant is exposed to various environmental stresses, drought (Asaad, 2013; Sundus, 2020), salinity (Mohmmad, 2019; Mohmmad and Asaad, 2019), radiation (Asaad, 2019) and heavy metals (Sabah, 2020; Sabah and Asaad, 2020). Proline gets rid of free radicals, as proline has certain properties that are more important in the stress caused by sulphur dioxide, as it reduced the increase in cellular pH resulting from the dissolution of sulphur dioxide in the water content of the cell. The accumulation and increase of proline in plants is exposed to stress by sulphur dioxide gas (Li et al., 2014; Seyyednejad, 2011). Ascorbite also regulates the oxidation chain of sulphates and thus prevents and stops the production of ROS. In many experiments conducted on the tomato plant, an increase in the synthesis of ascorbite was observed as a result of prolonged exposure

Table 6: Effect of timing and addition of sulphur dioxide SO_2 and trehalose on concentration of ascorbate content ($\mu g.g^{-1}$)

Hour H	*Trehalose mg.L^{-1}*	*SO$_2$ mg.m^{-3}*			*Mean trehalose ×H*
		0	*5*	*10*	
1	0	31.82	38.02	42.43	37.42
	50	27.20	32.83	35.15	31.72
	100	27.20	30.79	32.75	30.24
2	0	32.12	40.45	43.20	38.58
	50	26.46	39.74	40.38	35.52
	100	25.74	37.40	39.80	34.31
LSD. 0.05		11.107*			8.542 NS
		SO$_2$ mg.m^{-3} × H			
H		*SO$_2$ mg.m^{-3}*			*Mean H*
		0	*5*	*10*	
1		28.74	33.88	36.77	33.13
2		28.10	39.19	41.12	36.13
LSD. 0.05		8.496 *			3.668 NS
		SO$_2$ mg.m^{-3} × trehalose mg.L^{-1}			
Trehalose mg.L^{-1}		*SO$_2$ mg.m^{-3}*			*Mean trehalose mg.L^{-1}*
		0	*5*	*10*	
0		31.97	39.23	42.81	38.00
50		26.83	26.28	37.76	33.62
100		26.47	34.09	36.27	32.27
LSD. 0.05		9.42*			4.514*
Mean SO_2		28.42	36.53	38.94	
LSD. 0.05		4.514*			

to sulfur dioxide gas for a period of 45 days (Chauhan, 2015). Lycopene works to preserve the photosynthetic pigments from oxidative stress and is able to scavenge the single oxygen radical O_2, which is raised with increasing abiotic stress (Ramel et al., 2013). Spraying with trehalose sugar led to an increase in the activity of the enzymatic antioxidants (SOD, POD and CAT) and non-enzymatic antioxidants as shown in Tables 1-6. The antioxidant defense system plays an important role in plant performance and reduces the effects of biotic and abiotic stresses as it is able to overcome the toxic effect of oxygen and enhance plant tolerance to stress (Singh et al., 2010). The enzymatic antioxidants include superoxide dismutase (SOD), catalase (SAT), peroxidase (POD) and reactive oxygen species (ROS). In addition, the treatment was carried out using trehalose sugar for a higher resistance to stress by increasing the activities of CAT and SOD when compared with the corresponding controls (Aldesuquy and Ghanem, 2015). Trehalose treatment appears to be the most effective in counteracting the negative effects of stress, as trehalose acts as a direct and indirect scavenger of ROS (Stolker, 2010) that stimulates the plant to accelerate the rate of ROS production and in turn sends the signal to activate enzymatic antioxidants to scavenge ROS to counteract any oxidative stress. Previous studies have demonstrated the external role of trehalose in modulating SOD activity under stress conditions (Nounjan et al., 2012). CAT is one of the most important antioxidant enzymes and has the highest turnover rates among all enzymes (Garg and Manchanda, 2009). Several results of studies confirmed that CAT activity was increased by exogenous trehalose and under stress conditions (Ali and Ashraf, 2011). Proline has high hydrophilic properties, so it has the role of osmosis. In addition, it has compatible actions in the cytoplasm of the cell and without interfering with the cellular structure and metabolism during stress. Proline can act as a signaling molecule, leading to the modification of mitochondrial function as well as affecting cell proliferation by stimulating certain

genes (Szabados and Savoure, 2009). Accumulation of proline helps maintain membrane integrity by reducing lipid oxidation by scavenging free radicals and protecting oxidative potential (Ashraf and Foolad, 2007). In addition, proline has a role in improving drought tolerance through its role in regulating stress-protective proteins (Demiral and Turkan, 2004) and reduce lipid membrane oxidation (Khedr et al., 2003). Proline accumulation may enhance the enzymatic activity of proline synthesis and decrease the catabolism of enzymes (Alqarawi et al., 2014).

Conclusion

In the context of the results obtained, it was recommended to use trehalose sugar sprinkled on the shoots to reduce the damage caused by sulphur dioxide, as well as its role in stimulating the antioxidant system of tomato plants.

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