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The Sulfur Dioxide Absorption Capacity of Plants Tecoma Stans and its Effect on Antioxidants

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ABSTRACT

The current study used to prove the efficiency of *Tecoma stans* to sulfur dioxide gas (SO_2) was at a concentration of 10 mg·m^{-3} which is equal to 3.82 ppm for the period from summer exposure (May – June 2023) to reduce SO_2 thus reduce some gaseous pollutants that cause global warming and some air pollutants and know the effect of seasonal conditions to mitigate these pollutants. The physiological changes of the three replicates of study plant were observed through equal time periods daily for a period of seven days covered with polyethylene under controlled conditions represented by a greenhouse. The process was repeated three times between the three exposures provide rest periods for the plant for a week. During summer exposure, it was found that the concentration of flavonoids was significantly increased as compare to control (5.222 ± 0.27 mg/100 ml of extract) from 6.58 ± 0.43 to 6.24 ± 0.31 mg/100 ml of extract, but this concentration was increased after the third exposure into to 6.99 ± 0.29 mg/100 ml of extract. There was decrease in Tannins concentration after the second exposure (with concentration 1.5 ± 0.05 to 0.72 ± 0.01 µl/ml)), but this concentration was returned increased significantly to 1.36 ± 0.01 µl/ml) after the third exposure to SO_2. Also in The enzyme activities for peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) demonstrated varied responses to SO_2 exposure, and *T. stans* showing distinct patterns of enzyme activation. The effect of POD and CAT increased in plants which exposure into SO_2, whereas CAT play important role in inhibition of ROS.

Keywords: phytoremediation, pollution, antioxidants, flavonoids, tannins, catalase, superoxide dismutase, peroxidase.

INTRODUCTION

Air, the most essential element for all life on Earth, has been polluted to dangerous levels due to widespread urbanization. Air is considered polluted when there are more particles and chemicals in it than would be expected under normal circumstances (Lee et al., 2020a). Globally, increased levels of headaches have been linked to increased levels of air pollution. A recent report by the World Health Organization found that 91 percent of the global population lives in places where air pollution levels are too high (Zhang et al., 2020). Carbon monoxide, sulfur oxides, nitrogen oxides, volatile organic compounds, and particulate matter containing toxic metals are just a few examples of the many pollutants that pose a risk to health of human (Burns et al., 2020). Air pollution prevention efforts have received a lot of attention and resources (Zeng et al., 2019).

Some air pollution is caused by natural processes but the vast majority is caused by human activities (anthropogenic) like manufacturing, transportation, waste disposal, and farming. With its ability to bring about cardiovascular disease, stroke, lung cancer, and respiratory illnesses, air pollution has been named by the WHO (2014) as the greatest single environmental health risk in the world. Air pollution has many negative effects, one of which is global warming (He et al., 2017). Air pollution and climate change have a double whammy effect: reducing ecosystem productivity and future water availability (Lee et al., 2020). Reducing emissions of air pollutants is one

strategy for mitigating the negative health effects of pollution of air. Adsorption and catalysis, two physically and chemically complex processes, are frequently used to reduce pollution emissions. Due to the high initial investment and ongoing running costs associated with such procedures, they are also typically quite pricey (Cao et al., 2019; Burns et al., 2020). The pollutant air could also be cleaned up by various methods such as photo-catalysis, activated carbon adsorption, and houseplants can be used to effectively purify air within a building (Bhave and Yeleswarapu, 2020; Dhanabalan et al., 2020; Zhang et al., 2020).

SO_2 is a major air pollutant that mainly originates from the combustion of fossil fuels containing sulfur, such as coal and oil, and industrial processes like metal smelting and petroleum refining (Lu et al., 2020). Power plants, factories, and vehicles are significant sources of SO_2 emissions. SO_2 reacts with other compounds in the atmosphere to form particulate matter and acid rain, leading to negative impacts on human health and the environment (Seinfeld and Pandis, 2016). As of 2019, the global average concentration of SO_2 was approximately 1.5 ppb (parts per billion), with higher levels observed in industrialized regions and areas with extensive fossil fuel combustion, such as China, India, and Eastern Europe (Lelieveld et al., 2019). To reduce air pollution, air pollution abatement strategies must be implemented. Air pollutants' emission at source is controlled with various policies and laws which forced pollutant generators to attenuate their emissions (Singh and Verma, 2007). New technologies that are sustainable and ecofriendly are adopted in industries and automotive manufacturers (Agarwal et al., 2018). Additionally, emission control systems such as incineration, electromagnetic precipitation, and wet scrubbers that minimize the emission of harmful pollutants to atmosphere are used to curb the impact of air pollution (Singh and Verma, 2007). Still, issues particularly expensive engineering devices, machine failure, high maintenance cost, and low efficiency pose problems in the industries which causes insufficient technologies being implemented for air pollution control (Singh and Verma, 2007). Some technologies had been implemented to remove and eliminate inorganic and organic pollutant in air, water, and soil such as physical, chemical treatment, and microbial treatments (Adnan et al., 2014; Hadibarata and Kristanti, 2012; Kanthasamy et al., 2020; Lau et al., 2020; Salim et al., 2019). Compared with other conventional remediation technology, phytoremediation has advantages of cost-effective, easy to operate *in situ*, and eco-friendly treatment. Phytoremediation is a biological-based biotechnology that utilizes plants, and their collaboration microbes to accumulate, stabilize, or degrade an organic and inorganic pollutant in soil, water, and air pollutants (Lasat, 2001).

Phytoremediation can be employed in abating IAP like carbon dioxide (CO_2), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and ozone (O_3) (Weyens et al., 2015). As the well-known natural carbon sinks of CO_2, plants extract carbon dioxide in the air through photosynthesis and store in their plant organs for short or long period of time. Carbon dioxide deposited in the plants was either transformed to humus or stored (Weyens et al., 2015). Carbon sequestration is the storage of carbon dioxide in plants for a long duration (Sedjo and Sohngen, 2012). In addition to protecting against free radical damage, antioxidants help prevent the oxidation of amino acids and proteins and the modification of protein function caused by the interaction of lipid-derived carbonyls with proteins. A substance that may prevent the oxidation of other molecules is called an antioxidant (Gulcin, 2020). The family, Bignoniaceae, includes the genus *Tecoma*, which has 14 species of shrubs or small trees. Plants of this genus are commonly referred to as trumpetbushes. Plants in this genus are often known as trumpet bushes (Fernandes and Mankad, 2022; Khattab et al., 2022). Aims of the study finding plants that are efficient in removing or reducing SO_2 as air pollutants without harm on plant, ranging from trees to shrubs. In addition to reducing climate changes, these plants deal with sand dunes and sand storms, adding aesthetics to the area. Examining the effect of study gases on the studied plants through measurement of enzymatic and non-enzymatic activity (antioxidants) (SOD, POD, CAT, polyphenol, tannins and flavonoids).

MATERIALS AND METHODS

Study design

Data collection was carried out systematically to ensure accurate results. Six-months old *Tecoma stans* plant were obtained. A randomized complete design (RCD) was performed. The study was conducted in the summer. Total three replicates of *Tecoma* were included in the study. The plants were placed in the isolated room (0.5 × 2 × 2 m). It was covered with polyethylene. The SO_2 treatment (10 $mg \cdot m^{-3}$) which is equal to 3.82 ppm for the period from summer

exposure (May – June 2023) to reduce SO_2 was given for three weeks followed by intermitted rest period for one week. The control plants did not receive any gas exposure for given study periods (Table 1).

Preparation of sulfur dioxide gas

Sulfur dioxide gas was prepared by burning or melting sulfur with the presence of an abundance of oxygen according to the following Equation:

$$S + O_2 \rightarrow SO_2 \uparrow \quad (1)$$

Based on the Proust' law, 10 mg·m^{-3} of SO_2 was prepared by burning 5 mg of sulfur using electric incense burner for prepare 10 mg·m^{-3} inside the 1 m^3 equal to 3.82 ppm (Fahad and Abdullah, 2022) polyethylene-covered chamber with dimensions of 1.25 × 1.25 × 0.64 sized 1 m^3, which contained studies plants showed in Figure 1 Proust's law as follows:

$$\frac{M_{SO2}}{W_s} = W_{SO2} \times M_s \quad (2)$$

where: W_S – weight of sulfur, M_S sulfur molar mass (32 g/mol), W_{SO2} – weight of sulfur dioxide, M_{SO2} – sulfur dioxide molar mass (64 g/mol).

Various physiological parameters were measured, including flavonoids, tannins, phenols, superoxide SOD, CAT, and POD for antioxidants, and chlorophyll A, chlorophyll B, and β-carotene for pigments.

Evaluation of antioxidants

Evaluation of enzymatic antioxidant activity

About 1 gm of plant tissue was cut and crushed using ceramic mortar after addition of K_2HPO_4 (1 N) then filtrated using medical gauze and the filtrate was centrifuged at 4000 rpm for 30 minutes, then the supernatant was placed into tube and kept at low temperature until usage (Pitotti et al., 1994).

- Evaluation of POD.

The POD assay was carried out as per the method described by Putter (1974) modified by Malik and Singh (1980). Enzyme activity was

Table 1. Experimental design

Weeks	Treatment group (n = 6)	Control group (n = 6)	Plant material collection
1st week	Sulfur dioxide gas exposure	No exposure to SO2	Last day of exposure
2nd week	No exposure to SO_2		
3rd week	Sulfur dioxide gas exposure		Last day of exposure
4th week	No exposure to SO_2		
5th week	Sulfur dioxide gas exposure		Last day of exposure
6th week	No exposure to SO_2		--

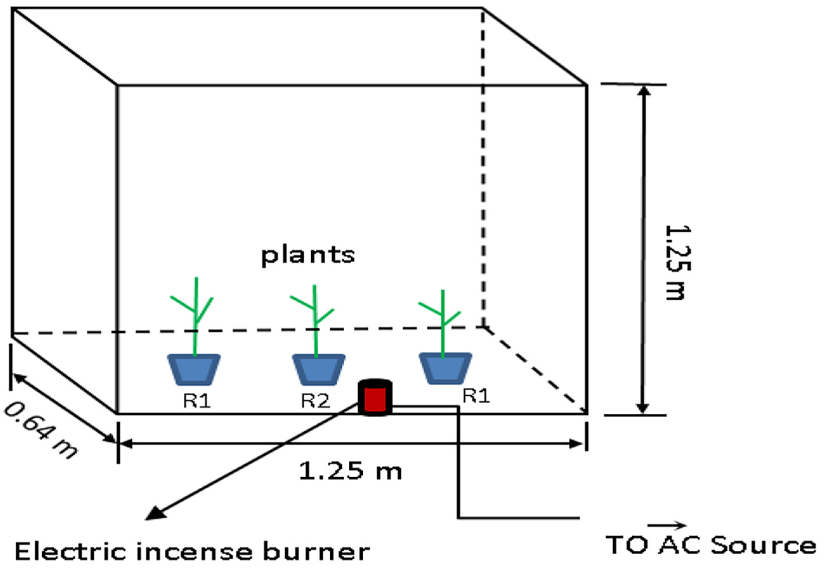

Figure 1. Chamber deigned of SO_2 gas

detected by increase in the absorbance at 436 nm. The activity of this enzyme per L of extract was estimated as the follows:

$$\text{Activity of enzyme } \left(\frac{\text{U}}{\text{L}}\right) = \\ = \frac{1000 \times 0.1 \times 3.18}{0.1 \times \Delta t \times 1 \times 6.39} = \frac{500}{\Delta t} \quad (3)$$

where: Δt was the absorbance of test material.

- Evaluation of catalase

The plant material was washed in 0.9% (w/v) NaCl solution. The material was homogenized using a glass homogenizer and ice cold 1.15% (w/v) potassium chloride solution. Homogenate plant solutions were then filtered and diluted (at a ratio of 1:500) with 0.05 M phosphate buffer for analyses of catalase activity, which were performed immediately. The activity of CAT was estimated as follows:

$$Catalase\ activity\ of\ test\ (kU) = \\ = \frac{2.303}{t} \times \log\frac{S^0}{S} \quad (4)$$

where: S^o – absorbance of the standard tube; S – absorbance of the tested tube.

The outcome activity was divided into number of grams of plant, which has been homogenized in one liter of extract (Hadwan and Kadhum, 2018).

Evaluation of non-enzymatic antioxidant activity

- Evaluation of total phenolic content activity

The reagent of Folin-Ciocalteau was utilized to quantify the total phenolic components in the extract after it had dried. Sample volume was 1 ml (1 mg/ml) and reagent volume was 1 ml. After waiting five minutes, 10 ml of 7% Na_2CO_3 was placed into the mixture, then deionized water (13 ml) was placed too. We let the mixture sit at 23 °C for 90 minutes. The mixture's absorbance was determined to be 760 nm (Khadabadi, et al, 2011). Following is an evaluation of the total phenolic content:

$$C = \frac{c \times v}{m} \quad (5)$$

where: C – Total phenolic content; m: pure tissue weight (g); v – extract volume (ml); c – gallic acid concentration

- Evaluation of total tannin content

A total of tannin (proanthocyanidin) content was determined using Catechin as a reference compound. 400 µl of extract was placed with 1.5 ml of concentrated HCl and 3 ml of (4% in methanol) vanillin. After fifteen minutes, the optical density was estimated at 500 nm. The result was displayed as g Catechin·10^{-1} DM (Broadhurst and Jones, 1978).

- Flavonoids' spectrophotometric estimation

A calibration curve was developed using different concentrations of quercetin, included 25, 50, 80, 100, 150, 200, 250, and 300 mg/100 ml in ethanol (80%). To prepare the standards, or samples, deionized water (2.8 ml), 1 mol/l potassium acetate (0.1 ml), 10% $Al(NO_3)_3$ (0.1 ml) and 95% ethanol (1.5 ml), were added to 0.5 ml of the standard solution or sample. When 10% $Al(NO_3)_3$ was called for, the same volume of deionized water was utilized instead. The reaction mixture absorbance was then estimated at 415 nm after incubation at temperature of room for thirty minutes (Ribarova et al., 2005). Quercetin was the universal symbol for flavonoids.

Statistical analysis

To determine the significance of the differences observed, a statistical analysis was conducted using the analysis of variance (ANOVA) method with a C.R.D. design. Subsequently, the least significant difference test (LSD) was employed to compare the means of different parameters, maintaining a probability level of 5%.

The following section will present the results and a comprehensive discussion of the data obtained from the experiments. This analysis will shed light on the effects of greenhouse gases on the selected plant species and contribute to our understanding of their adaptability in a changing environment.

RESULTS AND DISCUSSION

Maturing antioxidants in study plants that exposure to SO_2

Figure 2 represents the *Tecoma stans* plant before and after SO_2 exposure. Table 2 and Figure 2 showed the changes in POD, SOD and CAT activity after 1st, 2nd and 3rd SO_2 exposure.

The enzyme SOD, CAT, and POD activities demonstrated varied responses to SO_2 exposure in *T. stans* showing distinct patterns of enzyme activation. CAT activity was found to be decrease after 1st (0.59 ± 0.01 kU), 2nd (0.55 ± 0.00 kU) and 3rd (0.46

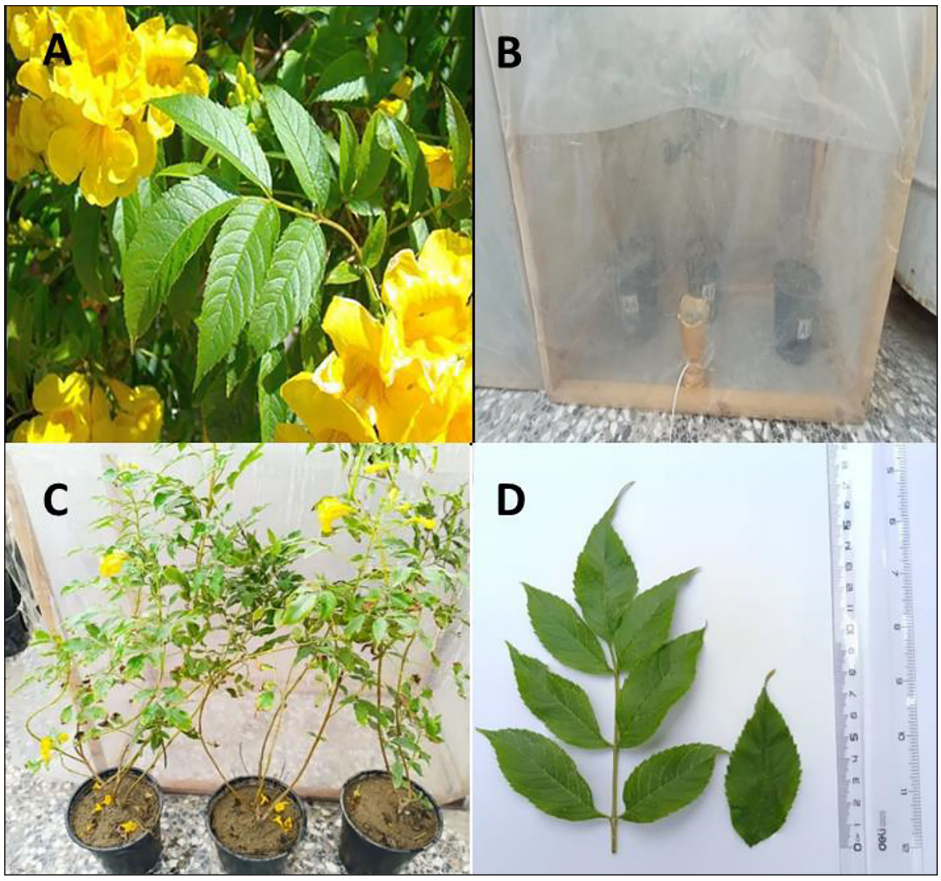

Figure 2. *Tecoma stans* plant before and after SO_2 exposure: A. *Tecoma stans*; B. *Tecoma stans* during exposure; C. *Tecoma stans* before exposure; D. Surface area of *Tecoma stans*

Table 2. The enzymatic antioxidant activity of *T. stans* after 1st, 2nd and 3rd exposure of SO_2

No	Summer exposure to SO_2 gas to *T. stans*	POD (EU/ml)	CAT (kU)	SOD (U/ml)
1	Control of *T. stans*	4.59 ± 0.12	0.411 ± 0.42	2.123 ± 0.17
2	First exposure to SO_2 gas	4.85 ± 0.95bc	0.59 ± 0.01abc	13.74 ± 1.21bc
3	Second exposure to SO_2 gas	3.7 ± 0.48bc	0.55 ± 0.00abc	15.55 ± 1.24ab
4	Third exposure to SO_2 gas	2.98 ± 0.15c	0.46 ± 0.01bc	16.93 ± 1.34a

± 0.01 kU) SO_2 exposure as compare to control (0.411 ± 0.42 kU) group. However, the activity was increase after first SO_2 exposure (0.59 ± 0.01 kU) as compare to control (0.411 ± 0.42 kU) group. Similarly, peroxidase activity was found to be decrease after 1st (4.85 ± 0.95 EU/ml), 2nd (3.7 ± 0.48 EU/ml) and 3rd (2.98 ± 0.1 EU/ml) SO_2 exposure as compare to control (4.59 ± 0.12 EU/ml) group. Superoxide dismutase (SOD) activity was found to be increase after 1st (13.74 ± 1.21 U/ml), 2nd (15.55 ± 1.24 U/ml) and 3rd (16.93 ± 1.34 U/ml) SO_2 exposure as compare to control (2.123 ± 0.17 U/ml) group.

The similar letters in the table showed non-significance difference in the value. Statistical significant was denoted by the different letters.

Evaluation of non-enzymatic antioxidant activity

Table 3 and Figure 3 represents the non-enzymatic antioxidant activity of *T. stans* after 1st, 2nd and 3rd exposure of SO_2. After first SO_2 exposure, flavonoid content (6.58 mg/100 ml) was found to be increase as compare to control (5.222 mg/100 ml). This indicates SO_2 gas has potential to synthesize flavonoids as a response to SO_2 stress. The "Second exposure" to SO_2 gas in exposure summer led to further increase in flavonoid content *T. stans* (6.24 mg/100 ml) as compare to control. However, the flavonoid content was less than the first exposure. This indicates the potential

inability to sustain flavonoid synthesis under prolonged SO_2 stress. This indicates that extended SO_2 exposure may disrupt flavonoid synthesis. In third SO_2 exposure resulted in an increase in flavonoid content for *T. stans* (6.99 mg/100 ml). Tannin content showed a significant decrease during "First exposure" to SO_2 gas (1.5 µl/ml) for *T. stans* as compare to controls (6.16 µl/ml). It further decreased in response to the "Second exposure" of SO_2 gas in *T. stans* (0.72 µl/ml). Tannin content increased during "Third exposure" of SO_2 gas (3.8133 µl/ml) in *T. stans* and highlighting a potential negative effect of prolonged SO_2 exposure on tannin production. The "First exposure" to SO_2 gas led to decrease in phenol content (12.32 mg/g) as compare to control (19.27 mg/g). In "Second exposure", the phenol content (14.79 mg/g) was increase as compare to first exposure (12.32 mg/g). However, it was still less than the control group (19.27 mg/g). Upon "Third exposure", the phenol content was further increase (25.21 mg/g) and it was found to be more than control plant. These findings highlight the complex interplay between phenols and SO_2 stress. The similar letters showed non-significance difference in the value. Statistical significant was denoted by the different letters.

Li and Yi (2020) reported significant increase in the SOD activity after 72 h of SO_2 exposure. Our results are accordance with this report. Increased in POD, CAT and SOD, leads to the formation of a powerful antioxidant defense system. Increased chloroplast-based sulphur assimilation in response to SO_2 stress was also associated with higher levels of the nonenzymatic antioxidants nonprotein thiol (NPT), glutathione (GSH), and cysteine (Cys), leading to a more efficient reactive oxygen species (ROS) scavenging system (Fujita and Hasanuzzaman, 2022).

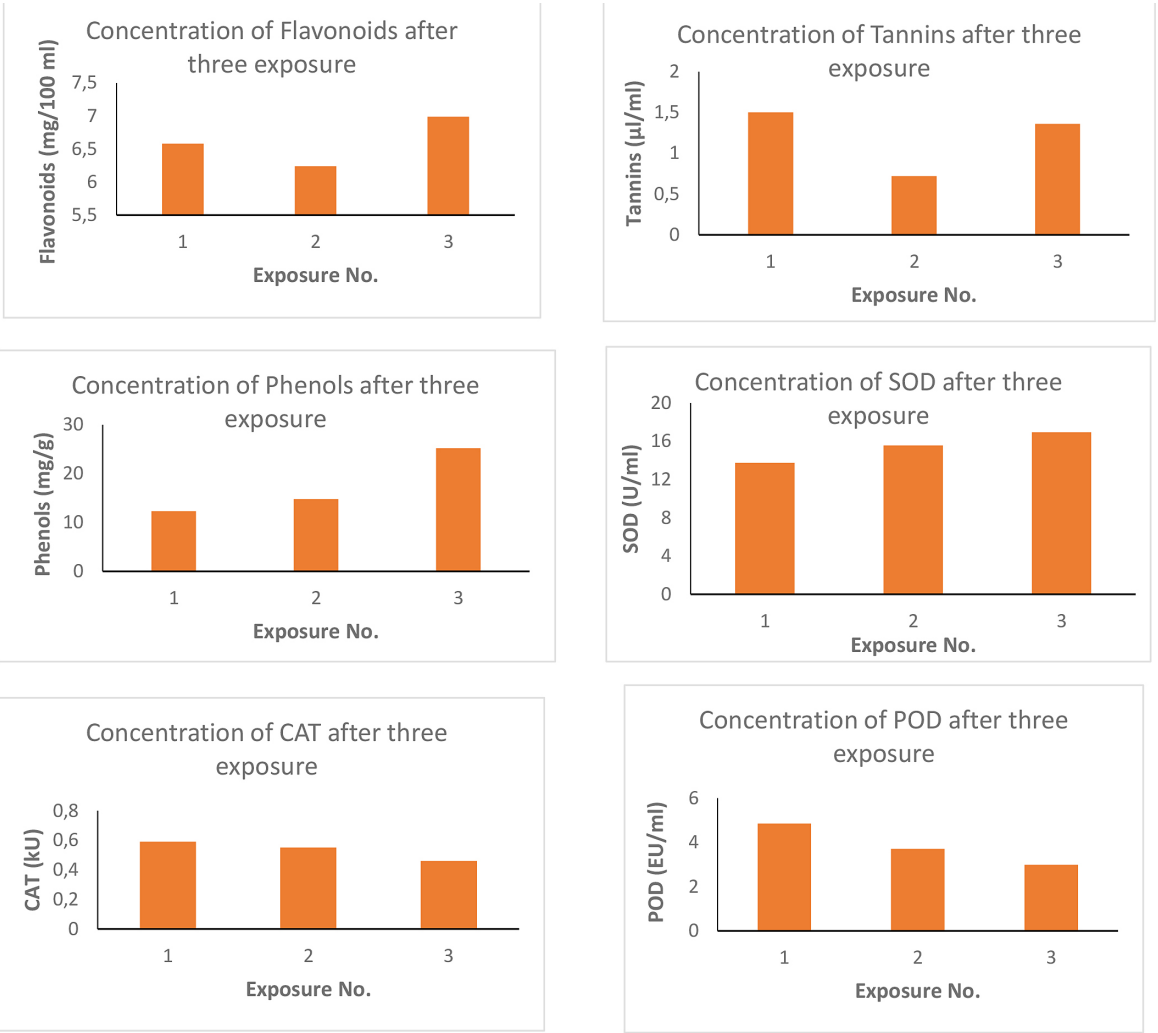

Figure 3. Showing levels of antioxidants: Flavonoids, tannins, phenols, SOD, CAT, and POD

Table 3. The non-enzymatic antioxidant activity of *T. stans* after 1st, 2nd and 3rd exposure

No	Summer exposure to SO_2 gas to *T. stans*	Phenols (mg/g)	Tannins (µl/ml)	Flavonoids (mg/100 ml)
1	Control of *T. stans*	19.27 ± 1.16	6.16 ± 0.97	5.222 ± 0.27
2	First exposure to SO_2 gas	12.32 ± 1.05c	1.5 ± 0.05abc	6.58 ± 0.43a
3	Second exposure to SO_2 gas	14.79 ± 1.24bc	0.72 ± 0.01c	6.24 ± 0.31a
4	Third exposure to SO_2 gas	25.21 ± 1.34a	1.36 ± 0.01bc	6.99 ± 0.29a

High SO_2 concentrations cause an increase in the accumulation of precursors of a few phenolic compounds, flavonoids, and condensed tannins in the needles and stem of Betula. It is suggested that plants that have sulfur-containing secondary metabolites reportedly utilize atmospheric SO_2 to produce them (Lahiri and Krishna, 2024). In response to SO_2 stress, plants use both enzymatic and nonenzymatic antioxidants to keep cellular redox homeostasis (Li and Yi, 2020). Increase SO_2 concentration leading to disrupt plastid membrane by relative ion leakage, in addition to accumulation of ROS, which lead to disrupt membranes in plants. Plant has defense mechanisms which work to eliminate oxidized compounds, whereas this effect increased with presence of SO_2 (Lee et al., 2017). The effect of POD and CAT increased in plants which exposure into SO_2, whereas CAT play important role in inhibition of ROS, while POD has limited role to eliminate SO_2 – that result from disruption of SO_2.

Understanding the effect of different greenhouse gases on plant antioxidant systems has significant environmental implications. These findings will give the impression about the increasing levels of greenhouse gases in the atmosphere. Our findings can help predict the potential impact on plant health and ecosystems. Plant Adaptation, our results suggest that different plant species exhibit varied responses to greenhouse gases and air pollution. This information can guide efforts to select and cultivate plant species that are more resilient to changing environmental conditions, ultimately aiding in ecosystem adaptation (Espeland and Kettenring, 2018). Greenhouse gases often co-occur with air pollutants. Our study provides insights into effect of these gases on the antioxidant capacity of plants, which can indirectly impact air quality and human health. The reduction in antioxidant capacity observed in some cases may lead to decreased plant resilience and compromised air quality (Karnosky et al., 2003; Sato et al., 2017). The research contributes to the existing body of knowledge in plant physiology and environmental science in several ways. The study demonstrated that different plant species respond uniquely to greenhouse gases, suggesting that species selection is critical when considering their role in climate change mitigation and ecosystem management (Species-Specific Responses). The responses of SOD, CAT, and POD to greenhouse gases varied across plant species and exposure periods. This highlights the intricate nature of antioxidant defense systems in response to environmental stressors. Based on the results of this study, we can able to provide few recommendations to the researchers. Species selection will be most important criteria. For the afforestation or reforestation planning projects, climate change mitigation, species selection showed greater impact. This can enhance the success of these projects. Secondly, encourage interdisciplinary research that brings together experts in plant physiology, environmental science, and atmospheric chemistry to gain a more holistic understanding of the effects of greenhouse gases.

CONCLUSIONS

In conclusion, this study provides valuable insights into *T. stans* respond to prolonged exposure to SO_2, through observing the concentration of enzymatic and non-enzymatic antioxidants and also the plant growth after exposure, especially after the third exposure, it find that the plant is efficient in removing sulfur dioxide. It is findings indicate that these responses are complex and species-specific, involving alterations in specific enzyme activity. These results have environmental implications, scientific significance, and offer direction for future research in the field of plant physiology and environmental science. By understanding the mechanism of plants adaptation to changing environmental conditions, we can develop strategies for climate change mitigation and ecosystem management.

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