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Original Research

An Analysis the Ability of *Acacia Glauca* Plant to Absorb Sulfur Dioxide and Its Impact on Its Pigments and Antioxidant

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Abstract:

The research focuses on how *Acacia glauca* produce chlorophyll pigments and antitoxins during winter seasons when exposure to SO_2 gas. The findings show that the plant react differently to SO2 exposure depending on the season and duration of exposure. In winter displayed varying levels of chlorophyll pigments with *Acacia glauca* demonstrating resilience compared to controls values. the exposure of *Acacia glauca* to SO2 cause non-significantly decrease in its concentration of chlorophyll A, whereas this concentration was decreased from 7.44 to 6.09 mg/g after the second while in third exposures increase 10.20 mg/g. while its concentration of chlorophyll b was range between (13.42 , 9.88 and 18.70 mg/g) respectively. Concentrations of carotenoid) were non-significantly decreased after second (from 20.87 b into 15.97 bc mg/g) and increase in third exposure (28.90 a) to SO_2.

tannin levels in *Acacia glauca* experienced a decrease after the exposure. Remained relatively unchanged in *Acacia glauca*. SO_2 exposure, superoxide dismutase (SOD) levels rose in plant with notable variations observed in multiple exposures for *Acacia glauca.* while POD levels decreased significantly in *Acacia glauca* after repeated exposures.

Keywords: *Acacia glauca*, SO2 absorption, Antioxidant synthesis, winter exposure, pigments,Catalase, peroxidase, flavonoid.

1. Introduction:

Environmental degradation (ED), pollution, climate change, and global warming have all garnered considerable interest from researchers, academics, and policymakers in the field of energy economics throughout the last several decades. Many studies, both empirical and theoretical, have looked at sustainable practice trends and different solutions (Danielle and Masilela, 2020). Climate change is one of the greatest

health concerns of the twenty-first century, according to the World Health Organization (WHO), and air pollution is their top environmental health worry (Campbell-Lendrum and Prüss-Ustün, 2019). Simultaneously, numerous commercial and industrial sectors have been able to fulfill their production and basic living needs thanks to the diverse resources offered by the natural environment. The necessity to treat the dangerous waste products of human activity, including carbon emissions, haze pollution, and different greenhouse gases, is one of the increasing pressures on the natural environment as stated by Nawaz *et al.* 2023). An increase in environmental degradation has been attributed to many developing economies' quest of faster rates of economic development (Chien *et al.,* 2021). Air pollution has been on the rise due to two main factors: the proliferation of modern industry and the increase in the global population (Burns *et al.,* 2020). According to Zeng *et al.* 2019, traditional power plants and motor vehicles are the main culprits responsible for air pollution. Vehicle exhausts contain a wide variety of pollutants, including carbon monoxide, volatile organic compounds (VOCs), nitrogen oxides (NO_x), particulates (soot of different sizes), and trace levels of heavy metals. Sulfur dioxide (SO_2), particulate matter (PM), and nitrogen oxides (NO_x) are among the air pollutants released by conventional power stations that produce electricity through the combustion of fossil fuels. A significant element in the warming of the atmosphere and the resulting climate change is carbon dioxide, which is generated in huge quantities by automobiles and factories (B. Zhang *et al.,* 2020).

Nitrous oxide, chlorofluorocarbons (CFCs), carbon dioxide, ozone, methane, and water vapor are the principal greenhouse gases (GHGs) that contribute to the increase in global temperatures. The lack of greenhouse gas emissions would cause the Earth's surface temperature to drop to an uninhabitable level. Because of this, understanding the Greenhouse Effect is crucial. Nevertheless, the rise in atmospheric quantities of greenhouse gases caused this disastrous occurrence, specifically, global warming (Al-Ghussain, 2019). Urbanization has caused lethal air pollution despite air being essential for life. Pollution occurs when air contains more chemicals and particles than usual. Lee *et al.* 2020] provided this data. Air pollution causes more headaches worldwide. According to a new WHO report, almost 90% of people on Earth live in locations with harmful air pollution. According to B. Zhang et al.[12] the study, toxic metal-containing particulate matter, sulfur oxides, nitrogen oxides, volatile organic compounds, and carbon monoxide can injure humans Guarneros, (Marco *et al.* 2020) Air pollution reduction measures have received a lot of attention (Zeng et al. 2019).

Updated WHO research suggests that 7 million people perished from air pollution in 2012, 2015. Air pollution is the major cause of preventable diseases such as lung cancer, cardiovascular disease, stroke, and respiratory illnesses, according to the WHO. Environmental damage from air pollution includes global warming. Air pollution and climate change reduce ecosystem productivity and water availability (Lee *et al.* 2020).

Plants are vital in reducing air pollution by soaking up and storing pollutants like SO2. Another way plants protect themselves from pollution-induced oxidative stress is by producing antioxidants.

Fahad and Abdullah ,2022) show that the plants were exposed to 0, 5, or 10 mg.m-3 sulfur dioxide gas daily for one or two hours. Plants were treated with 0,50 and 100 mg trehalose sugar sprays to reduce sulfur dioxide toxicity. Level 1. Increasing SO2 concentration and exposure increased the activity of enzyme antioxidants (SOD, POD, CAT) and non-enzymatic antioxidants (proline, vitamin C, lycopene), while trehalose spraying, especially at 100 mg, decreased antioxidant activity. Level 1. Reduced sulfur dioxide stress was another benefit

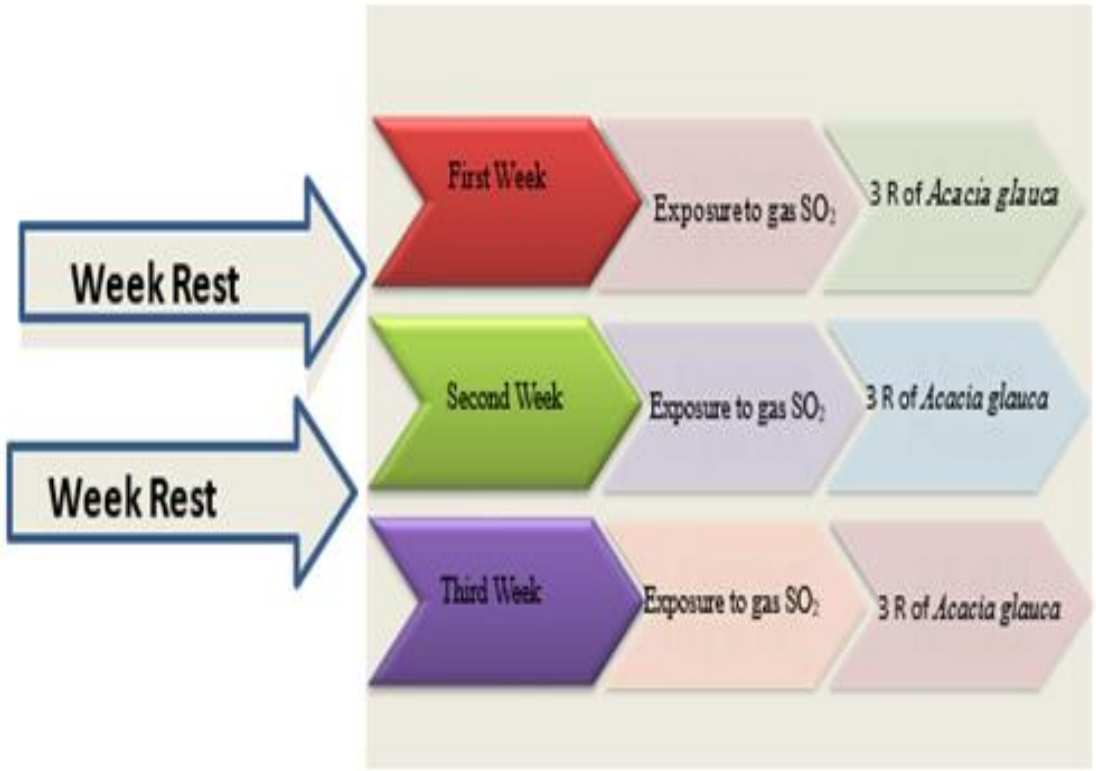
In Lee and colleagues 2020, reactive oxygen species (ROS), such as H2O2 and O2 - rise, causing oxidative damage to plants. Under high SO2 levels, plants' antioxidant defenses remove oxidative compounds more efficiently. Exposure to SO2 increases sulfur and sulfate content in plants, suggesting an oxidative process.

Additionally, cysteine, γ-glutamylcysteine, and glutathione levels drop. Previous studies found unique physiological, morphological, and biochemical responses to SO2 exposure in plants.

Antioxidants prevent free radical damage, oxidation of amino acids and proteins, and carbonyls from lipids from changing protein function. Antioxidants may prevent molecular oxidation. According to Gulcin 2020, an antioxidant "significantly delays or inhibits the oxidation of that substrate when present in low concentrations compared to that of an oxidizable substrate. The original definition was "any substance that delays, prevents, or removes oxidative damage to a target molecule (Gulcin, 2020)

Acacia glauca, a member of the Fabaceae subfamily Mimosoideae, is a Prosopis species. Despite the misunderstanding of synonymy, A. glauca is a good species with agreed-upon boundaries. A discussion regarding the ranks and nomenclature of the Acacia subgenera continues as molecular taxonomy illuminates its position in the genus (Pasiecznik, 2015 ;Adiamo et al.2020).

We collected samples of A. Glauca from areas with varying levels of SO_2 pollution. These plants then were exposed to controlled amounts of sulfur dioxide for observation purposes. By monitoring changes in gas concentration before and after exposure we can assess their effectiveness, in absorbing sulfur dioxide.



Methodology:

Dataset

Saplings *Acacia glauca* , were collected from Babel city's nurseries, Howat Al-zowhur and Barakat Al-Zahra'a.

In this randomized complete block design (RCBD) study (figure 1), in the winter. The plants were under gas for three weeks, with one week off between each exposure. rooms were made ready, with 2 m3 of polyethylene covering each chamber (0.5 * 2 * 2 m).

Figure 1. The design of the study, R: replicate.

Figure 2. *A. glauca* during exposure to SO_2

Figure 3 surface area of study plant

The Method:

Sulfur Dioxide Gas Preparation:

The following equation describes the process of producing sulfur dioxide gas by combining sulfur with oxygen during combustion or melting:

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

Under Proust's law, 5 milligrams of sulfur (S2) were burned in a two cubic meter polyethylene house containing *Acacia glauca* and Tecoma stans to produce 10 milligrams of SO2, 7.5 milligrams of SO2 were burned to produce 10 milligrams of SO2. Proust's law in this way:

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

The sulfur molar mass (MS) is 32 g/mol, the sulfur weight (WS) is 32 g/mol, the sulfur dioxide weight (WSO2) is 64 g/mol, and so on.

Chlorophyll A and B concentration:

A 1 g of plant tissue (soft leaf) was cut into small pieces then crushed using ceramic mortar with addition of 10 ml of 80% acetone in order to extract the chlorophyll pigment. The obtained mixture was placed in glass test tube for 20 hours in refrigerator (4 ºC) in dark place. Then the tube was mixed and left for 1 to 2 hours at same conditions. The volume was completed into 50 ml by addition of distill water then the tube was centerfugated at 3000 rpm for ten minutes. One to two drops of hydrochloric acid (0.1 N) were added into tube for filtration of mixture. The absorbance of filtrate was calculated utilizing spectrophotometer at 663 to 645 nm. The chlorophyll concentration was counted as follows (Parry *et al.*, 2014):

$$Chlorophyll\ a \left(mg/g\right) = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000 \times w} \times V$$

$$Chlorophyll\ b \left(mg/g\right) = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times w} \times V$$

$$Total\ Chlorophyll \left(mg/g\right) = \frac{20.2\ A_{645} - 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where:

- W: Sample fresh weight in gram
- V: The extract volume in milliliter.
- A: Light path length in cell (1 centimeter).

Carotenoid concentration

A 1 g of plant tissue (soft leaf) was cut into small pieces then crushed using ceramic mortar with addition of 10 ml of 80% acetone in order to extract the chlorophyll pigment. The obtained mixture was placed in glass test tube for 20 hours in refrigerator (4 oC) in dark place. Then the tube was mixed and left for 1 to 2 hours at same conditions. The volume was completed into 50 ml by addition of distill water then the tube was centerfugated at 3000 rpm for ten minutes. One to two drops of hydrochloric acid (0.1 N) were added into tube for filtration of mixture. The optical density of filtrate was calculated using spectrophotometer at 452 nm. The concentration of carotenoid was counted as the following formula:

$$Carotenoid\left(mg/g\right) = \frac{(1000\,A_{452} - Chl\,a\,\times 1.82 - Chl\,b\,\times 85.02)}{1000\,\times w}\times V\,\times 198$$

Where:

- A: absorbance
- V: final volume of the extract (50 ml).
- W: weight of plant leaves (1 g)

Assessing Enzymatic Antioxidant Activity

After adding 1 N of K2HPO4 to 1 gram of plant tissue, the mixture was ground and crushed using a ceramic mortar. The resulting filtrate was passed through medical gauze. After centrifugation at 4000 rpm for 30 minutes, the supernatant was transferred to a tube and stored at a low temperature until needed.

SOD Procedure

To get the indicators ready:

1. One liter of dilute water (D.W.) with 2.85 grams of Tris and 1.11 grams of Na2-EDTA to make Tris-EDTA buffer (8.2 pH).
2. A solution containing 0.252% pyrogallol was prepared by dissolving 0.06 ml of strong hydrochloric acid in 1 liter of dilute water.
3. Following these steps, each reagent was prepared:

Using the following formula, we determined the absorbance at 420 nm against an analogous solution both before and one minute after adding pyrogallol:

$$Inhibition\ of\ pyrogallol\ autoxidation\ (\%) = \frac{\Delta A_{\text{control}} - \Delta A_{\text{test}}}{\Delta A_{\text{control}}} \qquad (3)$$

$$SOD\ Activity\left(\frac{U}{ml}\right) = \frac{Inhibition\ of\ pyrogallol\ autoxidation\ (\%)}{50\ \%} \qquad (4)$$

POD Procedure

1. Before use, the reagents were kept at a temperature of 25oC.
2. The cuvette was filled with 0.1 mL of POD solution, 0.5 mL of guaiacol, and 3 mL of buffer solution.
3. After thoroughly mixing the solution, the cuvette was inserted into the spectrophotometer.
4. The time required (Δt) in minutes to elevate the optical density by 0.1 was estimated after the density was increased by 0.05 by watching the read.

Activity of enzyme (U/L) =	1000 x 0.1 x 3.18		500
	0.1 x Δt x 1 x 6.39	=	Δt

Assessing Non-Enzymatic Antioxidant Activity

Once the extract had dried, the total phenolic components were quantified using the Folin-Ciocalteau reagent. The sample had a one mg/ml concentration and a 1 ml reagent volume. Ten milliliters of 7% Na2CO3 were added to the mixture after five minutes, followed by thirteen milliliters of deionized water. For 90 minutes, we allowed the combination to sit at 23 degrees Celsius. The absorbance of the combination was found to be 760 nm. The following is a summary of the phenolic content analysis:

$$C = \frac{c \times v}{m} \qquad (6)$$

In what context: Total phenolic content (C), gallic acid concentration (c), extract volume (v) (measured in milliliters), and pure tissue weight (m) are all variables to consider. Khadabadi, et al,2011).

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Results and Discussion

Maturing pigments in plants exposed to SO2

Table 1 measurement of Pigments during exposure winter to SO2 gas in *A. glauca*

Winter exposure of *A. glauca* to SO_2 gas	**Chlorophyll A (mg/g)**	**Chlorophyll B (mg/g)**	**B-Carotenoid (mg/g)**	**Total**
First exposure to SO_2 gas	7.44 b	13.42 b	4.348 b	20.87 b
Second exposure to SO_2 gas	6.09 b	9.88 bc	3.99 b	15.97 bc
Third exposure to SO_2 gas	10.20 a	18.70 a	8.56 a	28.90 a
Control	5.42106	4.22316	1.2078	9.64422

The results in table (1) indicated that the exposure of Studies plant to SO_2 cause non-significantly decrease in its concentration of chlorophyll A, whereas this concentration was decreased from 7.44 to 6.09 mg/g after the second while in third exposures increase 10.20 mg/g.

while its concentration of chlorophyll b was significantly decreased after second exposure from 13.42 to 9.88 mg/g, while this concentration was non-significantly increased to 18.70 mg/g.

Concentrations of all pigments (chlorophyll A, B and carotenoid) were non-significantly decreased after second (from 20.87 b into 15.97 bc mg/g) and increase in third exposure (28.90 a) to SO_2.

the table (1) with the control, we find higher values, and this is due to the effect of exposure of the study plant to SO_2 cause increase in the pigments, this nearest with (Goyal *et al.,* (2020) attribute to the effect of this gas on carbon metabolism and chlorophyll contents, which in turn effect on the growth and production of plants. Also increased levels of SO_2 are known to have negative effects on chloroplast structure, photosynthetic pigments, and photosynthetic efficiency (Lee *et al.,* 2017; Duan et al., 2019). The long-term exposure to SO_2, even in their low concentrations, cause reducing the content of chlorophyll, whereas this content effected by the environmental stress (Qamar, F. (2023).). In their high concentrations, SO_2 disrupt the thylakoids' function and interfere with electron transfer chain (Vishen *et al.,* 2022). Also, decreasing the chlorophyll content may also attribute to the disruption of plastid membrane due to the toxic nature of SO_2 (Dubey et al., 2020).

Also this result similarity with Li and Yi (2020) reported that Arabidopsis leaves that were subjected to 30 mg/m3 SO_2 for 72 hours showed a considerable rise in the amounts of chlorophyll a, chlorophyll b, chlorophylls (ab), and total carotenoids, while the chlorophyll a/b ratio fell. The ratio of chlorophyll to carotenoids hardly budged. Taken together, these findings suggested that photosynthetic pigments were involved in SO_2-induced responses. In addition to protecting the photosynthetic machinery from oxidative damage caused by SO_2, the increased concentration of carotenoids may also act as antioxidants.

Table 2. measurement antioxidant in *A. glauca* during winter exposure to SO2 gas

winter exposure to SO_2 gas *of A. glauca*	**Flavonoids (mg/100 ml)**	**Tannins (µl/ml)**	**Phenols (mg/g)**	**SOD (U/ml)**	**CAT (kU)**	**POD (EU/ml)**
First exposure to SO_2 gas	3.6928 bc	1.9408 b	16.1958 a	19.5409 b	0.5913 c	3.9100 c
Second exposure to SO2 gas	3.5203 bc	1.3708 b	16.2214 a	19.9667 b	0.6246 c	4.1140 d
Third exposure to SO_2 gas	7.10	6.09	15.77	21.46	0.548	5.253
Control	2.99	1.4925	14.03	19.28163	0.693212	4.539

Based on the table (2), there were non-significant differences in flavonoid concentrations in study plant (A. glauca), during exposure to SO_2 . so range between 3.6928 bc to3.5203 bc mg/100 ml and increase in third exposure 7.10 mg/100 ml compare with control value 2.99

Also, there were non-significantly differences in levels of Tannins after the exposure to SO2. However, levels of tannins were significantly decreased from 1.9408 b into 1.3708 b µl/ml and non-significantly increased into 6.09 µl/ml after the second exposure of A. glauca into SO_2. the phenols' level was non-significantly increased from 16.1958 a into 16.2214 a mg/g and significantly decreased into 15.77mg/g after the second and third exposures into SO_2. There was a significant difference in SOD levels after the exposure

to SO2 gas, whereas these levels were increased from 19.5409 b into 19.9667 b U/ml. Also, no significant differences in SOD levels after third exposure 21.46 U/ml. No significant differences in CAT and POD levels after exposure of study plant to SO2. CAT range from (0.5913 c, 0.6246 c to 0.548). In A. glauca, the POD levels were significantly decreased from 3.9100 c into 4.1140 d EU/ml and non-significantly increased into 5.253 EU/ml after the second and third exposures into SO2, respectively.

This is compatible with Li and Yi (2020) reported SOD activity was increased and showed a significant increase after 72 h of SO_2 exposure. Together, the increased activities of POD, CAT and SOD, formed 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 NPT, GSH, and Cys, leading to a more efficient ROS scavenging system (Fujita and Hasanuzzaman, 2022).

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, 2023). In response to SO_2 stress, plants use both enzymatic and nonenzymatic antioxidants to keep cellular redox homeostasis (Li and Yi, 2020). Increase in 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 SO2, whereas CAT play important role in inhibition of ROS, while POD has limited role to eliminate SO_2 that result from disruption of SO2 in cytoplasm (Verma *et al.,* 2014).

Conclusion:

A. glauca showed more tolerant. Flavonoid levels remained stable, whereas tannin levels plummeted. SOD levels rose, with *A. glauca* showing greater variability after numerous doses. CAT and POD levels were declined significantly in *A. glauca* following the second exposure. tannin levels decreased, flavonoid concentrations remained consistent. Phenol levels notably increased in *A. Glauca*. The study suggests that A. Glauca have the ability to absorb SO_2 as shown by changes in antioxidant production and pigment concentrations depending on exposure and seasonal variations. These findings underscore the significance of examining how plants react to air pollutants like SO_2 as how specific plant species can aid in reducing air pollution while upholding antioxidant defenses. Future research could delve into the mechanisms behind these observed reactions evaluate the prolonged impacts of SO_2 exposure on plant health and ecosystem resilience and explore the benefits of utilizing A. Glauca in phytoremediation strategies, for combatting air pollution.

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