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Stress Analysis of *Amaranthus hybridus* L. and *Lycopersicon esculentum* Mill. Exposed to Sulphur and Nitrogen Dioxide

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ABSTRACT

The effects of SO_2 and NO_2 on stress-related enzymes in *Amaranthus hybridus* [AH] and *Lycopersicon esculentum* [LE] were studied. The plants were exposed to SO_2 and NO_2 generated by chemical reaction in a chamber for one hour every day for three weeks. After exposure, chlorophyll content, ascorbic acid, catalase, proline content, superoxide dismutase and peroxidase from leaf samples were assessed. Catalase contents decreased (AH: 84.31 ± 2.56 and LE: 50.52 ± 1.74) in the plants relative to control samples, which showed appreciable retention (AH: 98.59 ± 4.70 and LE: 91.84 ± 7.12). Plants showed more reduction in peroxidase due to exposure to NO_2 compared to values obtained in plants treated with SO_2. Meanwhile, lower values of ascorbic acid and chlorophyll contents were obtained in plants exposed to SO_2 and NO_2 gases. On the other hand, proline content increased after exposure to the gases. Morphological disorders, such as leaf browning, chlorosis and shrinkage of leaves were also noticeable. So, it is apparent that plants exposed to elevated SO_2 and NO_2 gases did not exhibit sufficient capacity to counteract the stress imposed by these gases.

Keywords: Air pollutants, *Amaranthus hybridus*, catalase content, *Lycopersicon esculentus*, nitrogen dioxide, plant stress, sulphur dioxide

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INTRODUCTION

Air pollution is a major challenge and a key contributor to climate change. It damages the ecosystem through enhanced fluctuations in atmospheric constituents,

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which can directly affect plants through their leaves or indirectly through soil acidification (Tripathi & Gautam, 2007). When exposed to airborne pollutants, most plants experience physiological and biochemical changes before exhibiting visible damage to leaves (Javed *et al*., 2009; Liu & Ding, 2008). Reduction in the cost of acquiring technology have led to the proliferation of industries and vehicles, thereby, increasing toxic gases and particulate matters in our environment (Agbaire & Esiefarienrhe, 2009; Joshi, Chauhan, & Joshi, 2009). Tiwari, Agrawal and Marshall (2006) suggested that sulphur dioxide (SO_2) and nitric oxides (NO_x) are major pollutants, whose concentrations are higher in urban than in suburban and rural areas. These have been worsened by rapid industrialisation through non-ecofriendly approaches (Uka, Hogarh, & Belford, 2017).

Plants show species-specific effects when exposed to harmful levels of air pollutant. The stress induced by gaseous pollutants have not been given sufficient attention compared with other abiotic factors, such as water, salinity, and herbicides. This paper contributes towards addressing that void in the light of increased concentrations of air pollutants due to industrialisation and climate change. Injury from gaseous pollutants ranges from visible markings on the foliage, reduced growth and yield, premature death of the plant, leaf injury, stomatal damage, premature senescence, decreased photosynthetic activity, disturbance in membrane permeability, and reduction of growth and yield in sensitive plant species (Tiwari *et al*., 2006; Black, Stewart, Roberts, & Black, 2007). Oxides of sulphur and nitrogen dissolve in rain water and ultimately enters the soil, change soil chemistry resulting in nutrient deficiency and reduces uptake by roots of plants (Agrawal, Singh, Agrawal, Bell, & Marshall, 2006; Agrawal & Deepak, 2003; Tankha & Gupta, 1992).

Air pollution can directly affect plants through leaves or indirectly via soil acidification with the potential of causing physiological and biochemical changes before exhibiting visible damage to leaves (Liu & Ding, 2008). These biochemical and physiological changes may include chlorophyll content, proline absorption, ascorbic acid, peroxidase and catalase contents. Burning of fossil fuels containing sulphur compounds will result in release of fine particulate sulphates and sulphuric acid mist (Ogunstein & Smith, 2007). In humans, SO_2 can aggravate existing respiratory diseases, such as asthma, bronchitis and emphysema as it constricts air passages making it difficult to breathe (Grahame & Schlesinger, 2009). NO_x, which is needed for the formation of nitrogen dioxide (NO_2), is produced during high temperature combustion of fossil fuels in electric power generating facilities, industrial operations, automobiles and chemical processing plants may affect a human's health by causing acute bronchitis or pneumonia and may reduce resistance to respiratory infections, such as chronic lung impairment (Ifeanyichukwu, 2002).

Green leafy vegetables Green leafy vegetables are crucial part of human diet (Oguntona & Oguntona, 1986). Generally, they are consumed as side dishes to major staples, such as cassava, cocoyam, guinea corn, maize, millet and plantains. Okoli, Nmorka and Unaegbu (1998) estimated that over 60 species of green leafy plants were consumed in Nigeria alone with an estimated *per capita* daily consumption of 65 g (Gruda, 2005). This includes plant species within families, such as *Amaranthaceae*, *Compositae*, *Portulacaceae* and *Solanaceae*.

Amaranth *Amarathus hybridus* [L.], Amaranthaceae is a traditional food plant in Africa. It has a potential to improve nutrition, boost food security, and support sustainable land care (NRC, 2006). Amaranth leaf is known in Swahili as *mchicha* (Olufemi, Assiak, Ayoade, & Onigemo, 2003). In Nigeria, it is a common vegetable known in Yoruba as *efo tete* or *arowo jeja*, *aleifo* (Hausa), and *Inine* (Igbo). Amaranth has been used widely by the Chinese for its healing chemicals to treat illnesses, such as infections, rashes, migraines and also used as fodder and dyes (Schippers, 2000). The leaves are also consumed as a nutritious leafy vegetable, being used both in cooking and in salads. These are excellent sources of vitamins, minerals, sugars and folic acid, which lower the risk of cancer and heart diseases (Steinmetz & Potter, 1996). They contain a variety of bioactive non-nutritive health promoting factors as antioxidants, photo-chemicals, essential fatty acids and dietary fibres (Mephba, Eboh, & Banigo, 2007; Negi and Roy, 2000).

Tomato (*Lycopersicun esculentum* [Mill.], Solanaceae) has many varieties that are now widely cultivated, sometimes in greenhouses and cool climates (Agbabiaje & Bodunde, 2002). Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. It is a perennial in its habitat, although often grown outdoors in temperate climates (Sheeja, Mondal, & Rathore, 2004). It is sensitive to water logging and flooding and prefers well-drained soils.

The objective of this research is to assess the effects of common urban air pollutants (SO_2 and NO_2) on *A. hybridus* [AH] and *L. esculentum* [LE]. This study highlights the non-visible effects of pollutants by measuring the levels of stress-related enzymes on these plants after exposure to SO_2 and NO_2 in a gas chamber. The changes in their biochemical components will produce visible morphological changes noticeable only as an after effect. By understanding the effects of these gaseous pollutants on chlorophyll, proline, ascorbic acid, superoxide dismutase and catalase contents, the effects of these enzymes can be predicted with greater accuracy. The economic viability of these vegetables crops makes them a suitable subject to understand the economic and ecological impacts of these gaseous pollutants. Therefore, our results will contribute towards understanding the effects of these air pollutants on the production of these vegetables vis-a-vis food security and sustainable development.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the Experimental laboratory of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.

Sources of Soil Samples

Top soil (0 – 10 cm depth) was collected from the Botanical Garden of Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. The physicochemical and microbial properties of the soil have been reported by Ogwu and Osawaru (2014). About 2 kg of the soil samples were transferred into forty experimental bowls perforate at the base and used for planting. Twenty bowls were used for each AH and LE.

Plant Material

Seeds of AH and LE were obtained from the previous harvest collection of a local farmer specialising in vegetable production in a community near Benin City, Edo State. The seeds were tested for viability and only viable seeds were selected and used for the study. Four seeds were sown per bowl in the experimental plots of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State and later reduced to one.

Producing Nitrogen Dioxide (NO_2) and Sulphur Dioxide (SO_2) Gases

Nitrogen dioxide was produced according to Lin, Xiao, Yuan, Choi and Chan (2005) herein summarised. About 50 g of lead nitrate was transferred into a Buchner flask with an air-tight cork. The flask was put on top of a tripod stand with wire gauze, the other end of the Buchner flask was connected with a glass tubing to the experimental chamber. After successfully setting up the apparatus, the Bunsen burner was ignited and the Buchner flask was heated gently. As lead nitrate decompose, NO_2 gas was steadily released into the experimental chamber through the glass tubing. After complete decomposition, the Bunsen burner was turned off and the stopper connecting the glass tubing and the chamber was locked to avoid reverse flow of the NO_2 gas.

Sulphur dioxide was produced according to Klemm and Talbot (1991), herein summarised. About 50 g of sodium sulphite was transferred into a Buchner flask and a hollow cork used to cover the flask. A burette filled with concentrated H_2SO_4 was directly connected to the cork with a stopper and clamped to a retort stand. The Buchner flask was connected to the experimental chamber using a thick rubber tube connected end-to-end. After setting up, the burette was gradually opened to flow into the Buchner flask and the reaction began. Subsequently, SO_2 gas released was channelled into

the experimental chamber through the connected rubber tub. At the end of the experiment, 100 ml of the concentrated H_2SO_4 was used.

$$H_2SO_{4\,(aq)} + Na_2SO_{3\,(s)} \longrightarrow Na_2SO_3 + H_2O_{\,(l)} + SO_{2(g)}$$

The NO_2 and SO_2 gases-exposure were carried out in an air tight wooden chamber of about 1.5 ft square. The gas generation set up was directly linked to the experimental chamber using sealed glass tubing with a stopper in-between the chamber and the glass tubing. The potted plants were kept in the experimental chamber few minutes prior to the initiation of gas generation. Once set up was complete, the generation of the gas was initiated and terminated after 30 minutes. The stopper was closed and locked to prevent reverse diffusion of the gas in the chamber. The plants were taken out of the gas chambers after exposure and later watered.

Measurement of SO_2 and NO_2 Concentrations and Exposure of Plants

The concentrations of generated gases (SO_2 and NO_2) were measured using aeroqual air monitoring kit (AeroQual model series 500, made in U.S.). Before gas generation, the background NO_2 and SO_2 concentrations in the Laboratory and chamber were recorded. The measurements were repeated immediately after generation of the gases (Table 1). The instrument used to measure the generated gas was Aeroqual air monitoring kit, with model number: Series 500, made in U.S.

The temperature of the experimental chambers was also measured before, during and after generation of the gaseous pollutants and exposure of the plants (Table 2).

Three bowls each of the pre-cultivated plants were placed in the chambers for 1 hour every day for three weeks, after which the plants were removed for biochemical analysis. The leaves of AH and LE used for this study were 3 and 4 weeks-old respectively.

Table 1
Concentration of SO_2 and NO_2 gases in laboratory and chambers before and after generation

	Gaseous pollutant	Laboratory room	Gas chambers
Before Generation	SO_2	0.00 ppm	0.037ppm
	NO_2	0.64 ppm	0.190 ppm
After Generation	SO_2	31.41 ppm	133.30 ppm
	NO_2	0.063 ppm	1.266 ppm

Table 2
Temperature (°C) of experimental chamber before and after one hour of gas generation without the plant

	Day	Pollutants	Temperature (°C)
Before exposure	Day 1	SO_2	18.8
	Day 1	NO_2	17.4
After exposure	Day 1	SO_2	26.1
	Day 1	NO_2	23.9
Before exposure	Day 2	SO_2	22.8
	Day 2	NO_2	22.6
After exposure	Day 2	SO_2	29.7
	Day 2	NO_2	28.6

Preparation of Leaf Samples for Biochemical Analysis

After the plants were removed from the gas chamber, leaves were detached from the stem to prevent translocation and avoid enzyme denaturing because higher temperatures denature enzyme and retard growth of plants. The leaves were transferred into polythene bags, placed in a cooler containing ice until analysis. The plant leaves were immediately subjected to extraction with chloroform and distilled water respectively. The acetone extract was analysed for its chlorophyll content while the distilled water extract was stored in the refrigerator at -4°C for antioxidant assay.

The study adopted method of Roe and Kuether (1943) to obtain the level of vitamin C content. Ascorbic acid was oxidized to dihydroascorbic acid by the action of 2,6-dichlorophenol indophenol. The dihydroascorbic acid was then hydrolysed to diketogluconic acid in a strong acidic medium. This forms an osazone and re-arranges to a stable reddish-brown product, which is measured colorimetrically at 500 nm.

The method of Misra and Fridovich (1972) was adopted to estimate Superoxide dismutase. Catalase (CAT) activity was determined based on the method of Cohen, Dembiec and Marcus (1970).

Estimation of Glutathione peroxidase was done by preparing a reaction mixture of 3.5 ml distilled water, 0.5 ml of 5 % pyrogallol solution, 0.25 ml of 0.147 M H_2O_2 solution and 0.5 ml phosphate buffer (pH 6.0). The mixture was equilibrated at 20°C for about 5 minutes and 0.25 ml of 2 N H_2SO_4 was added to stop the reaction after exactly 20 seconds. The optical density was measured at 420 nm against the blank solution (water).

$$= \frac{OD_{430nm}/30_{sec} \text{ x } Vt \text{ x } Df}{M \text{ x } V \text{ x } L \text{ x } y}$$

Where OD = Absorbance of test sample at 430 nM

Vt = Total volume of the reaction mixture

Df = Dilution factor

M = Molar extinction coefficient of purpurogallin 12.0 $M^{-1}cm^{-1}$

L = Light path, 1 cm

V = Volume of sample homogenate used

Y = mg of protein in tissue used

Proline determination was carried out based on the methods of Bates, Waldran and Teare (1973).

Total Chlorophyll content (TCh), Chlorophyll *a* and *b* was measured based on to Yadawa (1986).

Statistical Analysis

The experiment had used a completely randomised design in the field during cultivation and exposure in the laboratory. All analyses in this study were the means of three replicates and standard error of deviation. Statistical analysis was conducted to determine the level of significance of the stress induced on the AH and LE by NO_2 and SO_2 measured using one-way analysis of variance and Tukey-Kramer multiple comparison posthoc test on SPSS version 19. The analysis considered AH and LE as the independent variable and the gaseous pollutants (NO_2 and SO_2) as the dependent variables with statistical significance cut-off of P less than 0.05.

RESULTS

Chlorophyll Content

Chlorophyll *a*, *b*, and total chlorophyll contents of AH and LE leaves exposed to SO_2 and NO_2 are presented in Figures 1 and 2.

Chlorophyll content in leaves of both AH and LE was greater in control plant than SO_2 and NO_2 exposed plant. However, chlorophyll was higher at early growth stage.

Chlorophyll content of AH exposed to NO_2 showed a decrease (Figure 1). The decrease in the chlorophyll contents (chlorophyll *a, b* and total chlorophyll) of AH plants exposed to NO_2 was significantly higher ($P < 0.0001$) in the 4 weeks-old plants compared with the 3 week-old plants. Significant differences ($P < 0.0001$) were thus recorded in the chlorophyll contents of AH plants for control as against exposed. The AH exposed to SO_2 showed an increase in the chlorophyll contents in the 4 week-old plant compared with the 3 week-old plants. A slight increase was observed in the control for the 4 week-old plants compared with the 3 week-old plants.

Chlorophyll contents in LE plants exposed to NO_2 showed a comparative increase for the control in the 4 week-old plants compared with the 3 week-old plants (Figure 2). An appreciable increase was observed in the chlorophyll contents of LE for the 4 week-old plants exposed to NO_2. The LE exposed to SO_2 showed a significant increase for both the exposed and control of the 4 weeks-old plants compared with the 3 week-old plants.

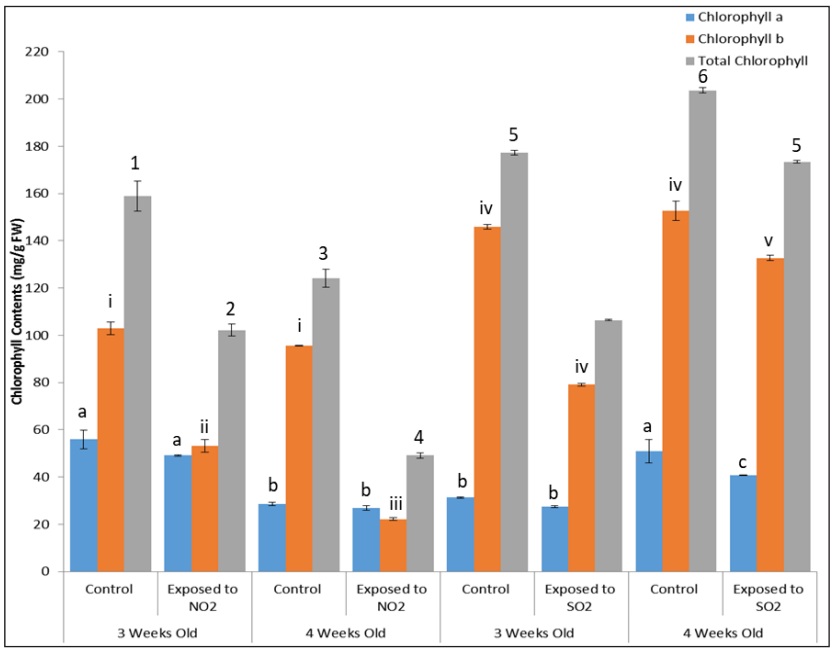

Figure 1. Chlorophyll content of *Amaranthus hybridus* plants exposed to NO_2 and SO_2 after 3 and 4 weeks. *Superscripts represents result of post-hoc test, where means sharing the same superscripts are not significantly different from each other.

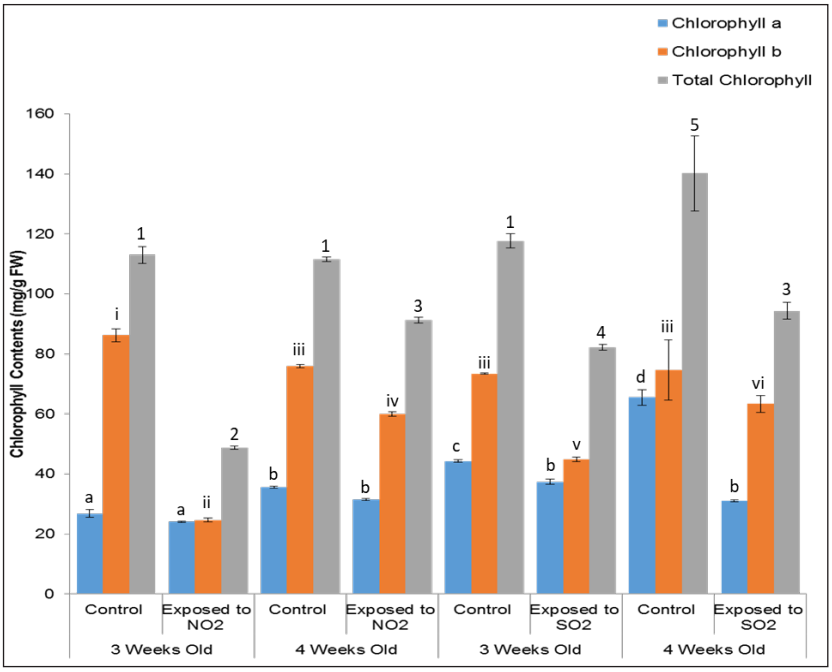

Figure 2. Chlorophyll content of *Lycopersicon esculentum* plants exposed to NO_2 and SO_2 after 3 and 4 weeks. *Superscripts represents result of post-hoc test, where means sharing the same superscripts are not significantly different from each other.

Catalase Content

The results obtained for the analysis of catalase content using leaf tissues of AH and LE is shown in Figure 3, where P value was significant for both three and four weeks-old plant exposed to NO_2 and SO_2 (at $p < 0.0001$). Catalase content of AH was shown to reduce more than 10 % due to exposure to NO_2 from 98.59 ± 4.70 to 84.31 ± 2.56 after three weeks and from 54.71 ± 10.80 to 36.57 ± 4.91 after four weeks. The catalase content of LE also dropped from 91.84 ± 7.12 to 50.52 ± 1.74 after three weeks and from 31.26 ± 1.28 to 22.61 ± 2.22 after four weeks. The effects of SO_2 on catalase content of AH suggested more than 50 % reduction after three and four weeks respectively. The effects of SO_2 on catalase content of LE suggested a more than 70 % reduction.

These results suggested catalase content was higher in the control than gas exposed plants. Also, it seemed that under stress, AH, tended to produce catalase as the major antioxidant enzyme. Catalase activities were very high in the leaf tissues of LE. Exposure to SO_2 and NO_2 probably caused reduction in catalase activities in AH.

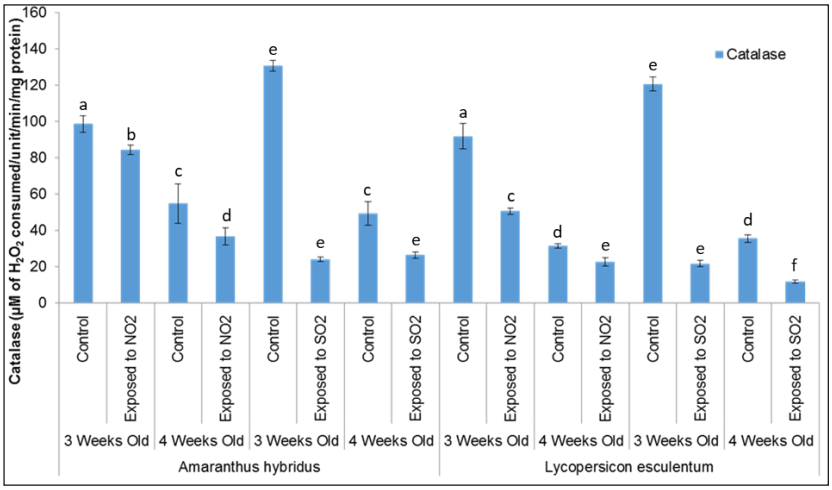

Figure 3. Catalase synthesis of *Amaranthus hybridus* and *Lycopersicon esculentum* plants exposed to SO_2 and NO_2 gases. *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other.

Superoxide Dismutase Content

The results obtained for the analysis of superoxide dismutase (SOD) contents using leaf tissues of AH and LE are shown in Figure 4. Superoxide dismutase content showed slight reduction after three and four weeks of exposure to SO_2 and NO_2 although these were significantly different with P value < 0.0001. The results indicate that the exposure to SO_2 and NO_2 caused reduction in SOD activities in the leaf tissues. A gradual decrease in SOD content was noticeable in the leaf tissues of LE exposed to SO_2 and NO_2 (after 3 and 4 weeks) compared with AH.

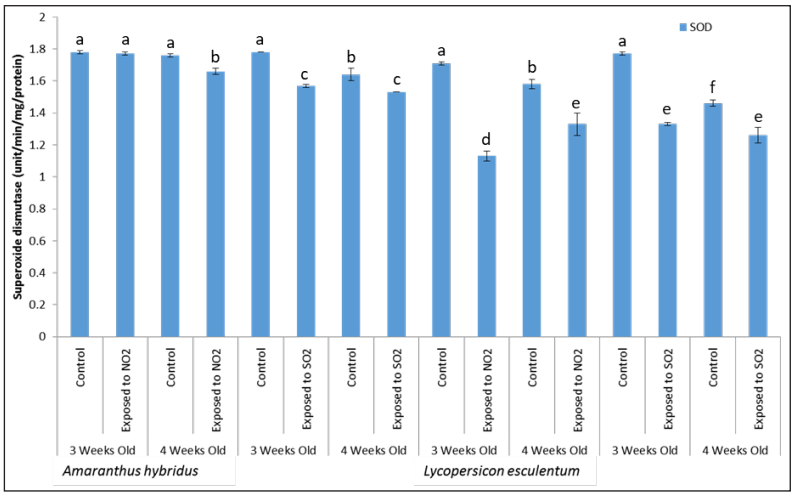

Figure 4. Superoxide dismutase synthesis of *Amaranthus hybridus* and *Lycopersicon esculentum* plants exposed to SO_2 and NO_2 gases. *Superscripts represents result of post-hoc test, where means sharing the same superscripts are not significantly different from each other.

Glutathione Peroxidase Content

The results obtained for the analysis of glutathione peroxidase content using leaf tissues of AH and LE are shown in Figure 5. Glutathione peroxidase activities were higher in LE plants in control conditions. In addition, exposure of plants to NO_2 did not affect glutathione peroxidase activities as such. It seems that under stress, glutathione peroxidase has a sharp reduction in the antioxidant enzyme activities of AH and LE plants exposed to SO_2 compared with NO_2 with respect to period of exposure to the gases.

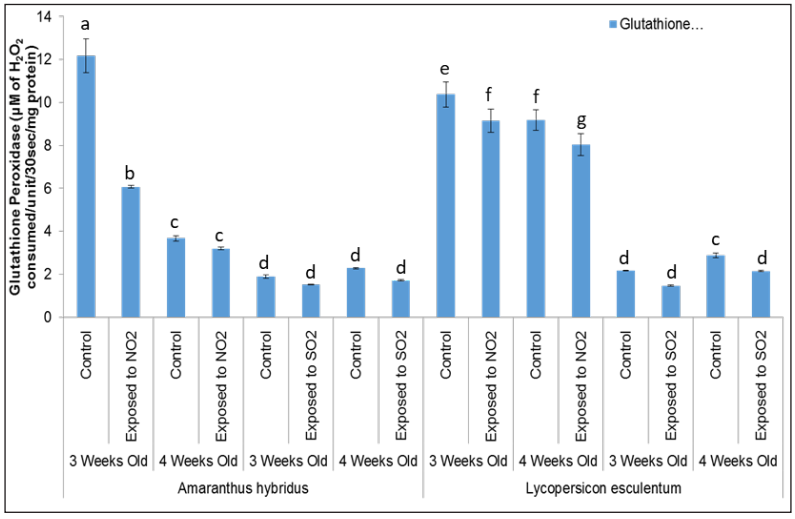

Figure 5. Glutathione Peroxidase synthesis of *Amaranthus hybridus* and *Lycopersicon esculentum* plants exposed to SO_2 and NO_2 gases. *Superscripts represents result of post-hoc test, where means sharing the same superscripts are not significantly different from each other.

Proline Synthesis

The exposure AH and LE to NO_2 and SO_2 stimulated the plants to increase the production of proline (Figure 6), which is an antioxidant compound. The increase in proline content is an indication of stress. It was observed that less amounts of proline were recorded for the older plants (4 weeks) exposed to the gases.

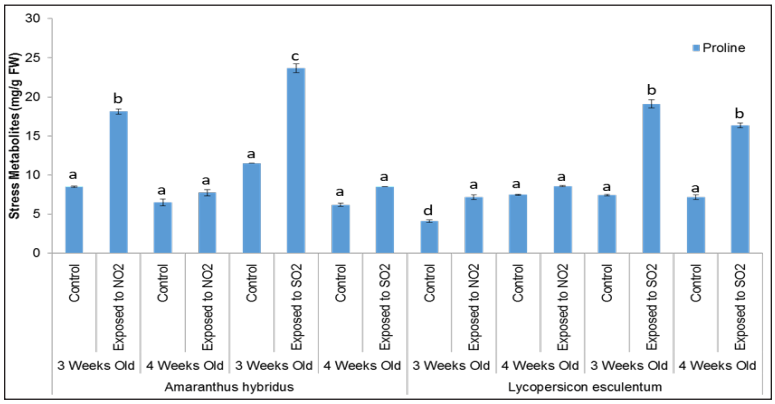

Figure 6. Proline synthesis of *Amaranthus hybridus* and *Lycopersicon esculentum* plants exposed to SO_2 and NO_2 gases. *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other.

Ascorbic Acid Content

Tables 3 and 4 shows the results for the ascorbic acid assay of AH and LE exposed to NO_2 gas after 3 and 4 weeks respectively. The controls of the ascorbic acid contents of the 3 weeks-old AH and LE plants were observed to have higher ascorbic acid contents than 4 weeks-old plants exposed to NO_2.

The results for the ascorbic acid assay of AH and LE *plants* exposed to SO_2 after 3 and 4 weeks) are shown in Tables 5 and 6. The study showed that the ascorbic acid contents were very high in the leaf tissues of AH controls compared to the exposed plants. More so, exposure to SO_2 caused a reduction in ascorbic acid activities. The consequences of this significant reduction in ascorbic acid activities were observed in the leaf tissues of LE plants exposed to SO_2 compared with the control plants.

Table 3
Ascorbic acid content of Amaranthus hybridus plants exposed to NO_2

Samples	Condition	*Age of plants	Vitamin C (mg/g FW)
Amaranthus hybridus	Control	3 weeks-old	28.13 ± 0.21^{a}
Amaranthus hybridus	Exposed	3 weeks-old	17.75 ± 0.79^{b}
Amaranthus hybridus	Control	4 weeks-old	11.73 ± 0.18^{c}
Amaranthus hybridus	Exposed	4 weeks-old	7.92 ± 0.71^{d}

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement; P value was < 0.0001; *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other

Table 4
Ascorbic acid content of Lycopersicon esculentum plants exposed to NO_2

Samples	Condition	*Age of plants	Vitamin C (mg/g FW)
Lycopersicon esculentum	Control	3 weeks-old	16.13 ± 0.05^{a}
Lycopersicon esculentum	Exposed	3 weeks-old	4.00 ± 0.20^{b}
Lycopersicon esculentum	Control	4 weeks-old	6.15 ± 0.39^{b}
Lycopersicon esculentum	Exposed	4 weeks-old	4.52 ± 0.07^{b}

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement; P value was < 0.0001; *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other

Table 5
Ascorbic acid content of Amaranthus hybridus plants exposed to SO_2

Samples	Condition	*Age of plants	Vitamin C (mg/g FW)
Amaranthus hybridus	Control	3 weeks-old	11.78 ± 0.05^{a}
Amaranthus hybridus	Exposed	3 weeks-old	8.30 ± 0.14^{b}
Amaranthus hybridus	Control	4 weeks-old	18.73 ± 0.15^{c}
Amaranthus hybridus	Exposed	4 weeks-old	9.61 ± 0.34^{b}

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement; P value was < 0.0001; *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other

Table 6
Ascorbic acid content of Lycopersicon esculentum plants exposed to SO_2

Samples	Condition	*Age of plants	Vitamin C (mg/g FW)
Lycopersicon esculentum	Control	3 weeks-old	6.10 ± 0.14^{a}
Lycopersicon esculentum	Exposed	3 weeks-old	4.13 ± 0.05^{b}
Lycopersicon esculentum	Control	4 weeks-old	13.18 ± 0.09^{c}
Lycopersicon esculentum	Exposed	4 weeks-old	3.48 ± 0.07^{b}

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement; P value was < 0.0001; *Superscripts represents result of posthoc test, where means sharing the same superscripts are not significantly different from each other

Morphological Changes

The morphological changes observed in the plants because of their exposure to the gaseous pollutants are presented in Table 7. The plants became weak and showed yellowing of leaves possibly as a result of chlorophyll degradation of chlorophyll. The leaves also shrunk.

The condition of the plants prior to exposure to the gaseous pollutants is shown in Figure 7. They appear healthy.

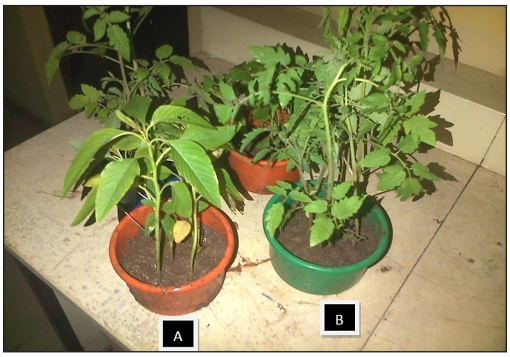

Figure 7. Amaranthus hybridus (A) and *Lycopersicum esculentum* (B) plants prior to exposure to NO_2 and SO_2.

Table 7
Morphological observations of Lycopersicun esculentum and Amaranthus hybridus plants after exposure

Plant species		SO_2		NO_2
Lycopersicun esculentum	1.	Plants appeared very weak as if it was suffering from drought	1.	Plants appeared very weak as if it was suffering from drought
Amaranthus hybridus	2.	Leaves appeared yellow probably due to rapid chlorophyll degeneration.	2.	Leaves appeared yellow probably due to rapid chlorophyll degeneration.
Lycopersicun esculentum	3.	Leaf shrinkage and stem weakening.	3.	Leaf shrinkage and stem weakening.
Amaranthus hybridus	4.	Severe leaf browning.	4.	Severe leaf browning.

After four weeks of exposure to NO_2, the plants appear shrunken and the leaves are no longer green (Figure 8).

Figure 9 shows the *Amaranthus hybridus* and *Lycopersicon esculentum* after four weeks of exposure to SO_2 gas.

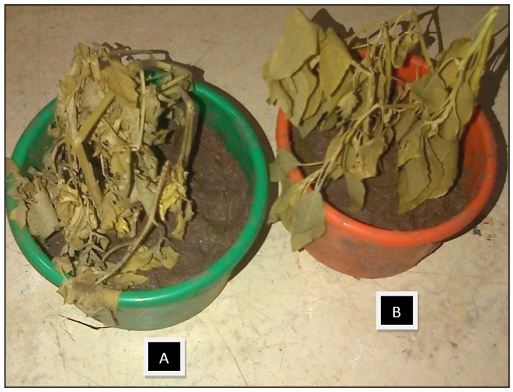

Figure 8. Lycopersicon esculentum (A) and *Amaranthus hybridus* (B) and plants after four weeks of exposure to NO_2

Figure 9. Lycopersicon esculentum (A) and *Amaranthus hybridus* (B) and plants after four weeks of exposure to SO_2

DISCUSSION

The results of this study clearly showed that NO_2 and SO_2 triggered the rapid induction of antioxidant stress enzymes in the leaves of AH and LE. Superoxide dismutase, catalase, glutathione peroxidase, ascorbic acid, proline and chlorophyll contents showed a reduction in the antioxidant enzyme levels when exposed to SO_2 and NO_2 gases. Therefore, inducing stress reactions capable of affecting metabolic pathway in chloroplasts, mitochondria, peroxisomes, plasma membrane, apoplast, endoplasmic reticulum, and cell-wall is vital (Sen, 2012). For instance, according to Woo, Lee and Lee (2007) NO_2 and SO_2 induced the excessive excitation energy within the chloroplasts, which ultimately resulted in increased

generation of reactive oxygen species (ROS) and **oxidative stress**. This may be responsible for the cell death observed in the present study due to oxidative damage to cellular macromolecules. Excess production of ROS due to environmental stress leads to progressive oxidative damage, which may cause cell death as the antioxidative defence mechanisms cannot scavenge the overproduced ROS (Sharma, Jha, Dubey, & Pessarakli, 2012). More so, the sessile nature of plants compounds this challenge from gaseous pollutants.

Increased activities of these enzymes and non-enzymatic antioxidants are considered indicators of oxidative stress in plants (Ruuhola, Rantala, Neuvonen, Yang, & Rantala, 2009). Peroxidase is an antioxidant enzyme whose activity in plants may be used as an indicator of gaseous pollutant to evaluate urban air pollution (Li, 2003). The report of Wu and Tiedemann (2002) showed that the increase of SOD, POD, CAT and APX was induced by the accumulation of singlet oxygen (O_2^{-1}) and hydrogen peroxide (H_2O_2). The results of this study showed that in response to air pollution, the activities of CAT, SOD, and GPx in AH and LE in leaves were decreased. However, an increase in proline content was recorded. This is similar to the study Seyyednejad, Niknejad and Yusefi (2009) where significant increase in proline content was recorded in *Albizia lebbeck*. The results of the present study are consistent with those of Tripathi and Gautam (2007) and Tiwari *et al*. (2006). They observed a decreased in chlorophyll and catalase level in leaves subjected to ambient air pollution, except for proline contents which increased during plant stress. More so, the changes in chlorophyll content, ascorbic acid content, SOD, GPx and CAT activities were significant ($p < 0.0001$) compared with the control.

The influence of different pollutants on plant is associated with oxidative damage at cellular level and the plants response to this oxidative stress by changes in the activities of ROS scavenging enzymes (Pukacka & Pukacki, 2000). Thus, increased anti-oxidative enzymes activity in the leaves is strongly associated with their resistance to air pollution (Woo & Je, 2006). However, the sensitivity to different pollutants differed between plants and within plant species. In addition, it has been reported that ageing leaves contain lower antioxidant levels (Wu & Tiedemann, 2002). Peroxidase activity varies with plant species and with the concentration of pollutants. Much experimental work has been conducted on the effects of air pollutant on crops and vegetation at various levels ranging from biochemical to ecosystem levels. When exposed to airborne pollutants, most plants experience physiological changes before showing visible damage to leaves (Liu & Ding, 2008). SO_2 and NO_2 are the most phytotoxic pollutants; these polluting gases enter leaves through stomata, following the same diffusion pathway as CO_2 (Streets & Waldhoff, 2000). The current study observed a reduction in catalase contents of the control as well as study plants. This reduction in catalase may be due to degradative processes

in the plant owing to photo oxidation. Brisson, Zelitch and Havir (1998) studied the effects of antisense on photorespiration, where the CO_2 compensation point at a leaf temperature of 38°C showed a significant linear increase with a reduction in catalase content. Plant catalase are known to be associated with protection against accumulation and toxicity of hydrogen peroxide [H_2O_2]. Since the peroxide contents of the environment was not measured prior to the study, reduction in catalase may be associated with accumulation of H_2O_2 by the study plants. MacRae and Ferguson (1985) posited that reduction in catalase maybe due to the inability of damaged peroxisomal membranes to transport catalase precursors into the peroxisome. Catalase also has several complementary biochemical roles within the plant, and these interactive actions may also account for the reduction observed in the study. Catalase isozyme is associated with mitochondria and in the cytosol (Scandalios, Guan, & Polidoros, 1997). In general, changes in catalase is likely due to the plant response to changes in environmental condition or biotic challenges (Mhanmdi *et al.,* 2010).

Ascorbic acid content is another parameter that may be used to decide the tolerance of plant to air pollution. It plays a significant role in light reaction of photosynthesis, activates defence mechanism under stress condition and functions as a powerful antioxidant (Arora, Sairam, & Sirvastava, 2002; Caviglia & Modenesi, 1999; Conkin, 2001).

Environmental, physiological and morphological tolerance mechanism to gaseous pollutants may be due to avoidance and stress tolerance through strain avoidance or tolerance (Taylor, 1978). The changes in these biochemical compounds as well as in their morphology suggest that the tolerance threshold of these two plant species exceeded the concentrations of toxic gaseous pollutants. To elucidate the tolerance levels of these plants to these gases, subsequent study may involve varied concentrations of NO_2 and SO_2 with considerations given to strain/species as well as biochemical and physiological limits of the tolerance. Plant tolerance may be also be affiliated with their physical structures, such as stomata, which serve as a potential entry point and the length of exposure to these pollutants. Anderson and Mansfield (1970) found that critical tolerance level to pollutants correlated with the soil concentration although this may vary.

Numerous studies indicate that oxidative stress due to atmospheric pollutants enhances the quantity of low molecular antioxidants such as ascorbic acid in cells. Ascorbic acid is a very important primary antioxidant which reacts with hydroxyl radical, superoxide and singlet oxygen, as well as secondary antioxidant. In this study, ascorbic acid level in polluted or exposed leaves of AH and LE decreased in response to air pollution stress. Species differed in the magnitude of response to pollutant exposure, although differences were not

consistent within taxonomic or functional groups (Honour, Bell, Ashenden, Cape, & Power, 2009). The current knowledge of effects of air pollution on plants is mostly based on experiments where plants have been exposed to high concentrations of air pollutants for short periods under experimental conditions. However, less is known about responses of plants to air pollutants at environmentally relevant concentrations and for long durations in field conditions (Li, 2003). It is well known that oxidative stress decreases the activities of antioxidant enzymes and produces many changes in plant morphological activities and growth as observed in the present study. Therefore, plant stress caused by these air pollutants is a costly process that induces plants to redirect useful resources to produce a cascade of biochemical, cellular, molecular and morphological responses to survive.

Jahan and Igbal (1992) emphasised that significant reduction in different leaf variables in a polluted environment was traceable to the constituent chemicals in the environment. In a study on SO_2 effect on *Platanus acerifolia*, changes in leaf blade and petiole size was observed (Dineva, 2004). In urban environments, trees play an important role in improving air quality by taking up gases and particles (Woo & Je, 2006). A change in vegetation may be indicative of the impacts of air pollution.

CONCLUSION

Exposure of AH and LE to SO_2 and NO_2 initiated a stress response that showed visible morphological changes. The energy required for these stress responses is costly to the plants. The decrease in chlorophyll, superoxide dismutase, catalase, glutathione peroxidase and ascorbic acid contents of the leaves may potentially affect normal growth and development of these vegetables. The marketability of these crops depends on the buoyancy of their leaves, which are affected by exposure to these gases. There is a need to regularly check the concentration of these gases in urban centres where these crops are cultivated in home gardens to meet the subsistence needs of the family.

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APPENDIX

Supplementary Results

Supplementary Table 1
Chlorophyll contents of Amaranthus hybridus plants exposed to NO_2

Samples	Condition	*Age of plants	Chlorophyll *a* (mg/g of Sample)	Chlorophyll *b* (mg/g of Sample)	Total chlorophyll (mg/g of Sample)
Amaranthus hybridus	Control	3 weeks-old	55.90 ± 3.86	103.00 ± 2.64	158.90 ± 6.30
Amaranthus hybridus	Exposed	3 weeks-old	49.16 ± 0.30	53.07 ± 2.70	102.23 ± 2.53
Amaranthus hybridus	Control	4 weeks-old	28.53 ± 0.76	95.57 ± 0.23	124.1 ± 3.84
Amaranthus hybridus	Exposed	4 weeks-old	26.87 ± 0.92	22.23 ± 0.49	49.10 ± 1.21

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 2
Chlorophyll contents of Lycopersicon esculentum plants exposed to NO_2

Samples	Condition	*Age of plants	Chlorophyll *a* (mg/g of Sample)	Chlorophyll *b* (mg/g of Sample)	Total chlorophyll (mg/g of Sample)
Lycopersicon esculentum	Control	3 weeks-old	26.77 ± 1.32	86.23 ± 2.12	113.00 ± 2.80
Lycopersicon esculentum	Exposed	3 weeks-old	24.03 ± 0.15	24.63 ± 0.64	48.67 ± 0.49
Lycopersicon esculentum	Control	4 weeks-old	35.57 ± 0.25	75.92 ± 0.59	111.49 ± 0.81
Lycopersicon esculentum	Exposed	4 weeks-old	31.37 ± 0.32	59.87 ± 0.76	91.23 ± 1.07

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 3
Stress enzyme contents of Amaranthus hybridus plants exposed to NO_2

Samples	Condi-tion	*Age of plants	Superoxide dismutase (unit/min/ mg protein)	Catalase (µM of H_2O_2 consumed/ unit/min/mg protein)	Glutathione peroxidase (µM of H_2O_2 consumed/ unit/30sec/ mg protein)	Proline (mg/g of Sample)
Amaranthus hybridus	Control	3 weeks	1.78 ± 0.01	98.59 ± 4.70	12.19 ± 0.79	8.50 ± 0.10
Amaranthus hybridus	Exposed	3 weeks	1.77 ± 0.01	84.31 ± 2.56	6.07 ± 0.07	18.13 ± 0.32
Amaranthus hybridus	Control	4 weeks	1.76 ± 0.01	54.71 ± 10.80	3.67 ± 0.1153	6.50 ± 0.44
Amaranthus hybridus	Exposed	4 weeks	1.66 ± 0.02	36.57 ± 4.91	3.19 ± 0.06	7.74 ± 0.42

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 4
Stress enzyme contents of Lycopersicon esculentum plants exposed to NO_2

Samples	Condition	*Age of plants	Superoxide dismutase (unit/min/mg protein)	Catalase (µM of H_2O_2 consumed/unit/min/mg protein)	Glutathione peroxidase (µM of H_2O_2 consumed/unit/30sec/mg protein)	Proline (mg/g of Sample)
Lycopersicon esculentum	Control	3 weeks	1.71 ± 0.01	91.84 ± 7.12	10.37 ± 0.59	4.10 ± 0.17
Lycopersicon esculentum	Exposed	3 weeks	1.13 ± 0.03	50.52 ± 1.74	9.15 ± 0.53	7.17 ± 0.29
Lycopersicon esculentum	Control	4 weeks	1.58 ± 0.03	31.26 ± 1.28	9.18 ± 0.49	7.47 ± 0.06
Lycopersicon esculentum	Exposed	4 weeks	1.33 ± 0.07	22.61 ± 2.22	8.03 ± 0.51	8.57 ± 0.06

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 5
Chlorophyll contents of Amaranthus hybridus plants exposed to SO_2

Samples	Condition	*Age of plants	Chlorophyll *a* (mg/g of Sample)	Chlorophyll *b* (mg/g of Sample)	Total chlorophyll (mg/g of Sample)
Amaranthus hybridus	Control	3 weeks	31.30 ± 0.20	146.00 ± 1.00	177.30 ± 0.92
Amaranthus hybridus	Exposed	3 weeks	27.40 ± 0.36	79.06 ± 0.50	106.47 ± 0.15
Amaranthus hybridus	Control	4 weeks	51.00 ± 4.94	152.67 ± 4.16	203.67 ± 1.09
Amaranthus hybridus	Exposed	4 weeks	40.70 ± 0.17	132.67 ± 1.16	173.37 ± 0.55

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 6
Chlorophyll contents of Lycopersicon esculentum plants exposed to SO_2

Samples	Condition	*Age of plants	Chlorophyll *a* (mg/g of Sample)	Chlorophyll *b* (mg/g of Sample)	Total chlorophyll (mg/g of Sample)
Lycopersicon esculentum	Control	3 weeks	44.27 ± 0.40	73.40 ± 0.27	117.67 ± 2.42
Lycopersicon esculentum	Exposed	3 weeks	37.33 ± 0.85	44.80 ± 0.79	82.13 ± 1.00
Lycopersicon esculentum	Control	4 weeks	65.47 ± 2.57	74.67 ± 10.03	140.14 ± 12.48
Lycopersicon esculentum	Exposed	4 weeks	31.07 ± 0.35	63.30 ± 2.72	94.37 ± 2.87

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 7
Stress enzyme contents of Amaranthus hybridus plants exposed to SO_2

Sample	Condition	*Age of plants	Superoxide dismutase (unit/min/mg protein)	Catalase (μM of H_2O_2 consumed/unit/min/mg protein)	Glutathione peroxidase (μM of H_2O_2 consumed/unit/30sec/mg protein)	Proline (mg/g of Sample)
Amaranthus hybridus	Control	3 weeks	1.78 ± 0.00	130.67 ± 3.06	1.89 ± 0.07	11.50 ± 0.00
Amaranthus hybridus	Exposed	3 weeks	1.57 ± 0.01	24.00 ± 1.28	1.52 ± 0.02	23.67 ± 0.58
Amaranthus hybridus	Control	4 weeks	1.64 ± 0.04	49.13 ± 6.50	2.28 ± 0.02	6.20 ± 0.20
Amaranthus hybridus	Exposed	4 weeks	1.53 ± 0.00	26.24 ± 1.75	1.72 ± 0.029	8.50 ± 0.00

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001

Supplementary Table 8
Stress enzyme contents of Lycopersicon esculentum plants exposed to SO_2

Sample	Condition	*Age of plants	Superoxide dismutase (unit/min/mg protein)	Catalase (μM of H_2O_2 consumed/unit/min/mg protein)	Glutathione peroxidase (μM of H_2O_2 consumed/unit/30sec/mg protein)	Proline (mg/g of Sample)
Lycopersicon esculentum	Control	3 weeks	1.77 ± 0.01	120.67 ± 3.79	2.16 ± 0.02	7.40 ± 0.10
Lycopersicon esculentum	Exposed	3 weeks	1.33 ± 0.01	21.49 ± 1.75	1.47 ± 0.02	19.10 ± 0.52
Lycopersicon esculentum	Control	4 weeks	1.46 ± 0.02	35.45 ± 2.10	2.88 ± 0.11	7.17 ± 0.29
Lycopersicon esculentum	Exposed	4 weeks	1.26 ± 0.05	11.72 ± 0.84	2.15 ± 0.02	16.33 ± 0.29

Key = mean ± *S.D* (n = 3), * = Period of exposure before measurement
P value is < 0.0001