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Stress Measurements of *Solanum melongena* L. and *Celosia argentea* L. plants exposed to SO_2 and NO_2 gases separately in Chambers

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Abstract
Stress induces protection responses from exposed plants. Celosia argentea L. and Solanum melongena L. plants were separately exposed to NO_2 and SO_2 gases in chambers and their morphological appearance and biochemical responses were measured. Parameters analysed were morphological appearance, chlorophyll content, superoxide dismutase, catalase activity, peroxidase activity, proline and ascorbic acid contents. Three weeks and four weeks old C. argenta and S. melongena were separately exposed to NO_2 and SO_2 gases. After exposure, the morphological changes recorded for test plants (C. argentea and S. melongena) exposed to gas treatments were severe bleaching of leaves, dehydration, discolouration and wilting. Chlorophyll content analysis of leaves showed a significant reduction ($p< 0.05$) for test plants in both 3 weeks and 4 weeks old plants. Chlorophyll contents of C. argentea and S. melongena plants showed similar pattern where values for control plants were significantly higher than test plants ($p< 0.05$) under both NO_2 and SO_2 gases. Biochemical analysis for SOD, catalase, peroxidase and ascorbic acid contents showed the same pattern where values for control plants were higher than values for test plants ($p< 0.05$). Proline contents of leaves were significantly higher in test plants than in unexposed plants under NO_2 and SO_2 gases. The increase in proline contents by test plants is insufficient to give protection and survival to C. argentea and S. melongena exposed to NO_2 and SO_2 gases.

Keywords: Sulphur dioxide, nitrogen dioxide, stress, superoxide dismutase, proline

Introduction
The atmosphere of an environment supports plant's growth, reproduction and perpetuation in that environment. Industrial development increased the concentration of some gases e.g SO_2 and NO_2, changing the chemical composition of gases which may become detrimental to plants. Pollution has a significant impact on the species richness of plants found in a specific area; as increased levels of pollutants cause decline in species richness. However, these effects depend on the type of pollutant and plant group, i.e. trees, shrubs, grasses and herbs (1).
The concentrations of SO_2 and NO_2 are influenced by industrialisation and expansion of cities, increase in traffic, rapid economic development and high fossil fuel utilisation. The growth of both industrial and residential areas in many developing countries like Nigeria lack systematic planning, thus complicating air pollution challenges. At the regional level, the impact of air pollution on different local plant species is a major ecological issue. Overtime, scientists and environmentalist emphasize monitoring of air pollutants as a prerequisite for air quality control (2, 3, 4, 5, 6). Monitoring using biological indicators is simple, cheap and convenient and gives a clearer state of the local environment. The effects of gaseous pollutants on plants increase with concentration exposure, time and type of gases. Plant exposure to individual pollutant can be used to assess the long term quality of air and provide warning signals on species disappearance, reproduction and degradation.
Nitrogen dioxide (NO_2) is a reddish-orange-brown gas with an irritating acrid, characteristic pungent odour (7, 8, 9). It is corrosive, highly oxidizing (9) and noncombustible (10). Nitrogen dioxide can be generated by natural and anthropogenic sources. Nitrogen oxides are precursors of both acid precipitation and ozone, each of which is blamed for injury to plants. Nitrogen oxide combines with photochemical oxidants to form smog.
Sulphur dioxide SO_2 is a colourless and, non-flammable gas (11). Its odour has been described as sharp, pungent (8, 12, 13), choking (12), strong (11) and irritating (13). Sulphur dioxide is generated by natural or anthropogenic sources. Sulphur dioxide is taken up by leaves in gaseous form through the stomata. SO_2 presence in mesophyll combines with water to form acidic media that cause damage to organelles and components of cells. Unlike in higher plants, the absence of protective cuticle in mosses and lichens structurally, has made them to be extremely sensitive to monitoring of gaseous pollutants and application as biomonitors (14) . Sulphur dioxide on the other hand, can also modify the response of plants to other environmental stresses both biotic and abiotic, often exacerbating their adverse impacts (15). *Celosia argentea* Linn. is a vigorious, broadleaf annual belonging to the *Amaranthaceae* family. It is grown successfully in temperate as well as tropical regions. It is indigenously and traditionally cultivated in countries of Central and West Africa and is one of the leading leafy

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green vegetables grown in Nigeria, where it is known as "soko yokoto' meaning 'make husbands fat and happy' (16). *Celosia argentea* grows rapidly from seed depending upon the variety and soil fertility (17). Medicinal uses of *Celosia argentea* include its use for treatment of abscesses, cough, colic, diabetes mellitus, diarrhea, dysentery, eczema, eye problem, gonorrhea, menstruation problems, snake bites and wounds (18).

Solanum melongena Linn. commonly called egg plant is a tropical perennial plant belonging to the family *Solanaceae* (19). The plant is native to the India subcontinent grown extensively in Bangladesh, China, Pakistan and Philippine (20). It has been cultivated in southern and eastern Asia since prehistory. It is also cultivated in southern and eastern Nigeria extensively. The fruit is botanically classified as a berry and contains numerous small, soft seeds which are edible but have a bitter taste because they contain nicotinoid alkaloids and are grown mainly for food and medicinal purposes (21). Various parts of the plant are useful in the treatment of inflammatory conditions, cardiac debility, ulcer of nose, asthma and cholera (22). *Solanum melongena* is cultivated from the seed.

The objective of the study was to measure some stress indicators after exposure of plants to NO_2 and SO_2 gases separately. These indicators will suggest individual plant response to the gases involved.

Materials and Method

Plant materials

The plants used in this study were *Celosia argentea* and *Solanum melongena*. Seeds of these plants were collected from a vegetable grower in Iyowa community near Oluku, Edo State. These seeds were grown in experimental pots for four weeks. Three and four weeks old plants were used in this study.

Generation of NO_2 gas

Principle: Lead (ii) nitrate releases nitrogen dioxide gas when heated to temperature of about 100^oC.

$$Pb(NO_3)_{2(s)} \longrightarrow PbO_{(s)} + 2NO_{2(g)} + {}^{1}/_{2}O_{2(g)}$$

A 50g weight of lead (ii) nitrate was accurately measured into a clean Buchner flask and stopper with airtight cork. The lead (ii) nitrate was heated for one hour for complete decomposition. On complete decomposition, NO_2 gas was released and subsequently channeled into the experimental chamber using the glass tubes connected end to end.

Generation of SO_2 gas

Principle: Sodium sulphide releases sulphur dioxide gas when concentrated tetraoxosulphate (vi) acid is added to it.

$$H_2SO_{4(aq)} + Na_2S_{(s)} \longrightarrow Na_2SO_{4(aq)} + H_2O + SO_{2(g)}$$

A weight of 50g sodium sulphide was accurately measured into a Buckner flask and a cork with a hole at the center was used as a cover. A burette filled with concentrated H_2SO_4 was directly connected to the cork with a stopper and clamped to a retort stand. SO_2 gas subsequently released was channeled into the experimental chamber using the connecting rubber tubes end to end. At the end of the experiment, 50ml of concentrated H_2SO_4 was used.

Exposure of plant to SO_2 and NO_2 gases

The exposure of plants to SO_2 and NO_2 gases generated was carried out in air-tight chambers, constructed locally. Before the commencement of SO_2 and NO_2 generation, the environmental concentrations of these gases were recorded within the chamber and laboratory environment. This was called the background concentration of these gases. The instrument used for this measurements were Air Quality Monitoring kits (Aeroqual model no: 500, made in U.S) capable of detecting and quantifying atmospheric gases. The gases were generated for two hours continuously and terminated. Measurements of SO_2 and NO_2 concentration in the chambers after generation were taken. Thereafter, the plants were put in the chambers for two-hour exposure period for the first and second day. There were chambers for control and exposed plants. After the exposure period for the first day, plants were taken out of the chambers and brought back to the field. The same plants where taken to the laboratory for exposure on the second day after gas generation. After this second exposure, the plants where taken for biochemical analysis

Biochemical Assays

After 2 hours exposure of the plants to either SO_2 or NO_2 gas on the second day, leaf samples were harvested from the test and control plants respectively. The leaf samples used for chlorophyll content estimation were stored in chloroform while leaf samples for antioxidant determination were stored in distilled water and refrigerated.

Estimation of vitamin C

The method of Roe and Kuether (23) was adopted.

Enzyme extract preparation

The fresh leaf samples were removed from the refrigerator and kept on the laboratory bench for air-drying to reduce the wetness of the leaf tissues. Half a gramme (0.5g) of leaf sample was ground with 5ml 0.9% saline solution in a mortar and pestle into a paste. The paste was then centrifuge at 4000 rpm for 10 minutes. The

supernatant, which is the tissue extract, was decanted into specimen tubes for storage in the refrigerator. The tissue extract was used for estimation of SOD, catalase and peroxidase activities.

Estimation of superoxide dismutase (SOD, EC 1.15.1.1) activity

This is determined by the method of Misra and Fridovich (24, 25).

Estimation of catalase (CAT, EC 1. 11.1.6) activity

Catalase activity estimation was carried out using the procedure of Cohen *et al.* (26).

Estimation of peroxidase (POX, EC 1. 11.17) activity

This was carried out using the Andy and Goodman (27) methods, a modification of Chance and Maehly (28) method.

$2\text{pyrogallol} + 3H_2O_2 \xrightarrow{\text{POX}} 5H_2O + \text{purpurogallin} + CO_2$

The appearance of purpurogallin is measured at 420nm using spectrophotometer.

Proline content determination

Proline estimation was carried out according to the methods of Bates *et al*. (29) as modified by Marin *et al*. (30).

Determination of chlorophyll content

Chlorophyll a and b determination was carried out according to Dere *et al*. (31). The amount of chlorophyll pigment of each sample was calculated according to the formulae of Lichtenthaler and Wellburn (32).

$C_a = 11.75\ A_{662} - 2.350\ A_{645}$

$C_b = 18.61\ A_{645} - 3.960\ A_{662}$

Chlorophyll content = $C_a + C_b$

C_a = chlorophyll a, C_b = chlorophyll b

Data Analysis

Data obtained were used to calculate mean and standard deviations. T-test analysis was carried out to compare values obtained for exposed and control plants.

Results

The results obtained in this study are shown in Table 1 and Figures 1-8. Table 1 shows morphological features of plants after the second day of gaseous treatments. Plants brought out from the control chambers showed normal growth without any morphological abnormalities but the test plants showed severe bleaching and discoloration of leaves, dehydrated and wilted. The observations recorded were common to all plants from both chambers.

Table 1: Morphological observation recorded after exposure of *C. argentea* and *S. melongena* to NO_2 and SO_2 gases separately in chambers

Plant species	Morphological observations recorded after exposure to NO_2 and SO_2 gases separately	Inference
Celosia argentea and *Solanum melongena*	1. Plants appeared very weak as they were subjected to drought.	1. This means that the effect of the gases on the plants is like that of drought
	2. Leaves appeared yellow.	2. Rapid degeneration of chlorophyll molecules suspected.

Figure 1 shows chlorophyll a contents of leaf samples of *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases in different chambers. Values obtained for control plants were higher than that of treated plants. Four weeks old *S. melongena* plants gave higher values than 3 weeks old plants. Differences in chlorophyll a values between control and gas exposed plants were significant ($\alpha = 0.05$). Figure 2 shows values recorded for chlorophyll b when *C. argentea* and *S. melongena* plants were exposed to NO_2 and SO_2 separately. Similarly, values recorded for control were higher than gas exposed plants. Total chlorophyll content of *C. argentea* and *S. melongena* plants are shown in Figure 3. Higher values were recorded for plants under control conditions. Differences in values between control and gas exposed plants were significant ($\alpha = 0.05$). Figure 4 shows SOD activities recorded for *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases. The values recorded showed decrease in SOD activity for gas exposed plants. Figure 5 shows catalase activities recorded for *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases. Lower values were recorded for test plants. Differences in these values were significant ($\alpha = 0.05$). Figure 6 shows peroxidase activities in *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases. Also, lower peroxidase enzyme activities were recorded for test plants. Figure 7 shows proline content recorded for *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases. The values obtained indicated that the test plants induced higher accumulation of proline molecules. This is an indication that the plants exposed to gases were under stress. Differences in values were significant ($\alpha = 0.05$). Figure 8 shows the ascorbic acid content recorded for *C.argentea* and *S. melongena* plants exposed to NO_2 and SO_2 gases. Lower values were recorded for test plants. There were no accumulations of ascorbic molecules in test plants. Ascorbic acid also serves as antioxidant molecules in plants.

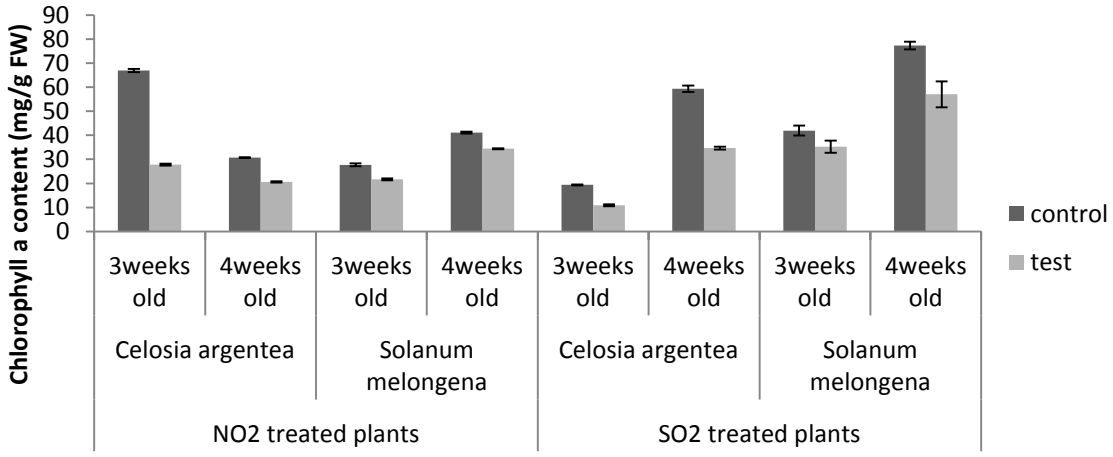

Figure 1: Chlorophyll a (mg/g FW) of leaf samples harvested from *Celosia argentea* and *Solanum melongena* exposed to NO_2 and SO_2 gas in a chamber.

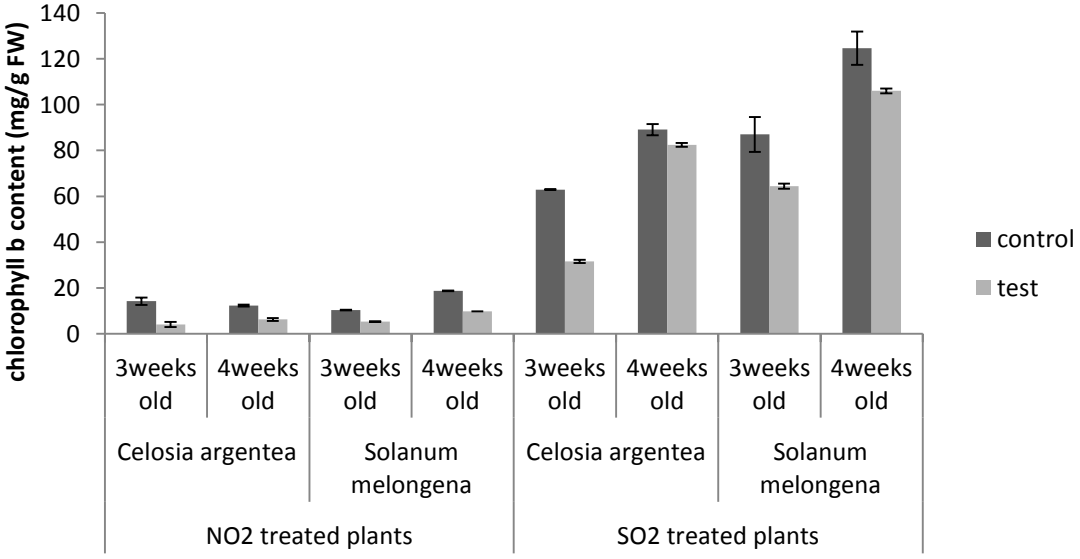

Figure 2: Chlorophyll b (mg/g FW) of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber

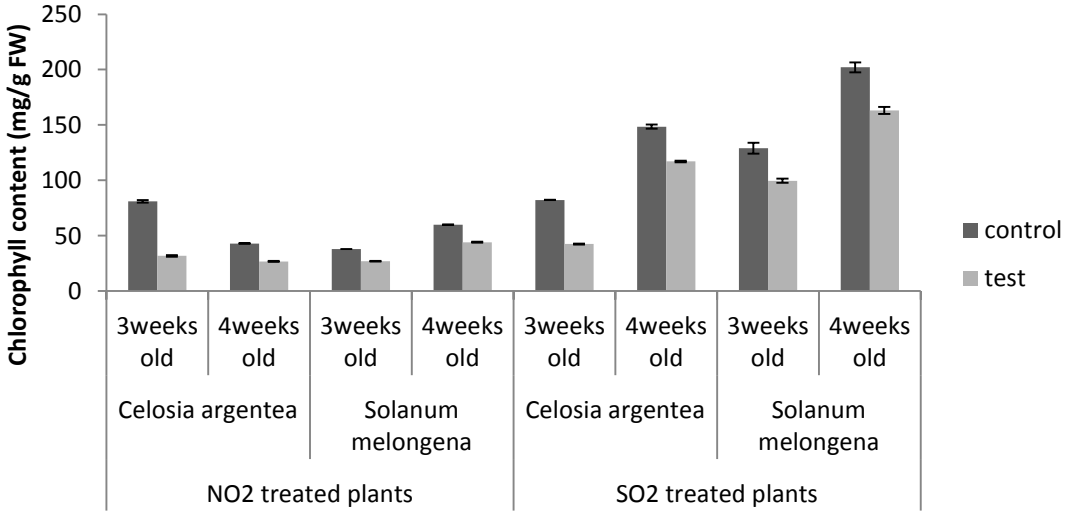

Figure 3: Chlorophyll content (mg/g FW) of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber.

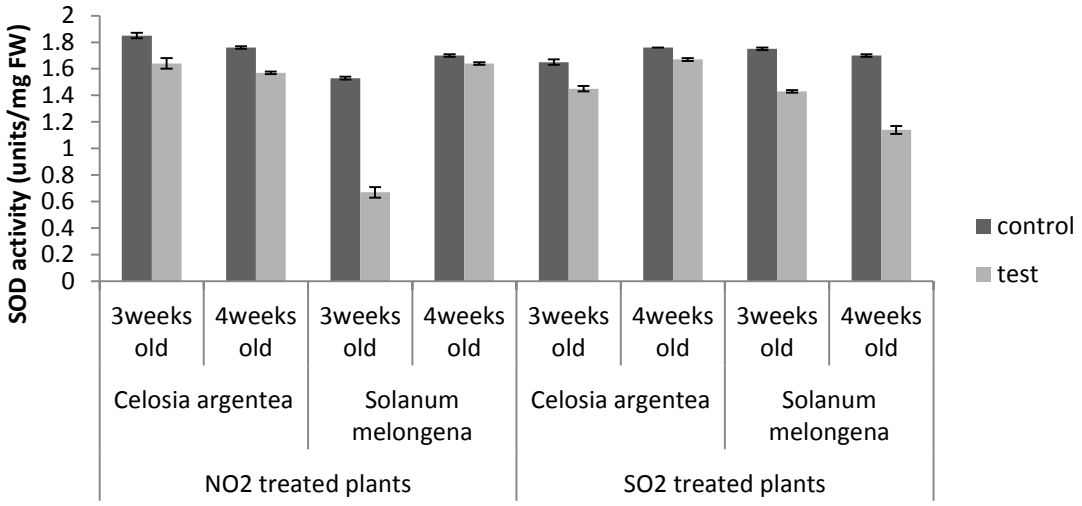

Figure 4: Superoxide dismutase activity (SOD) of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber.

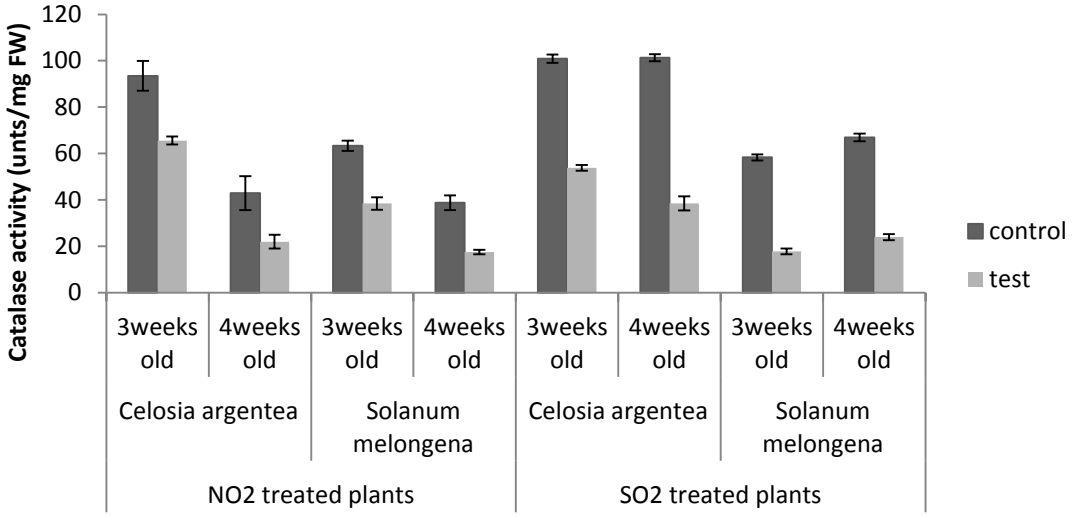

Figure 5: Catalase activity of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber.

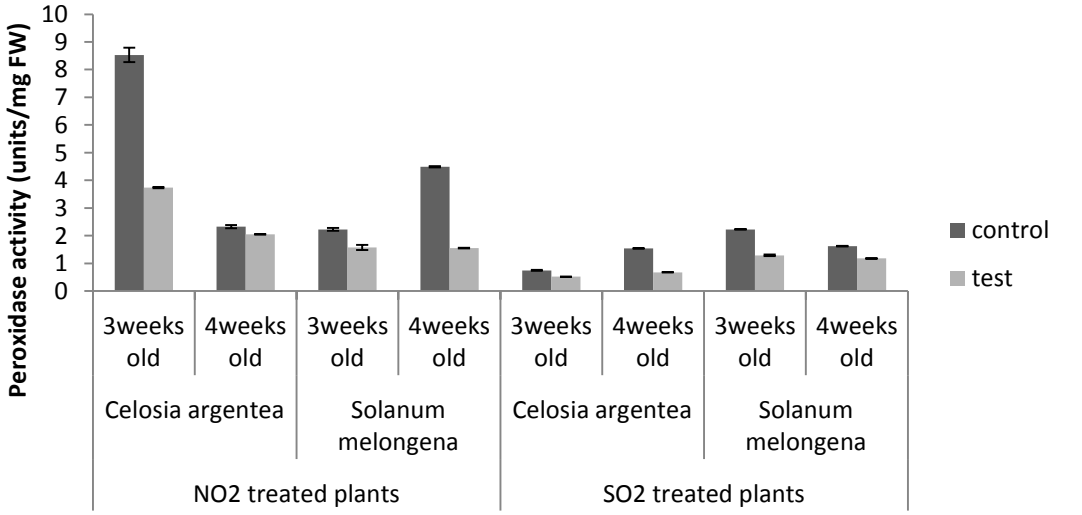

Figure 6: Peroxidase activity of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber

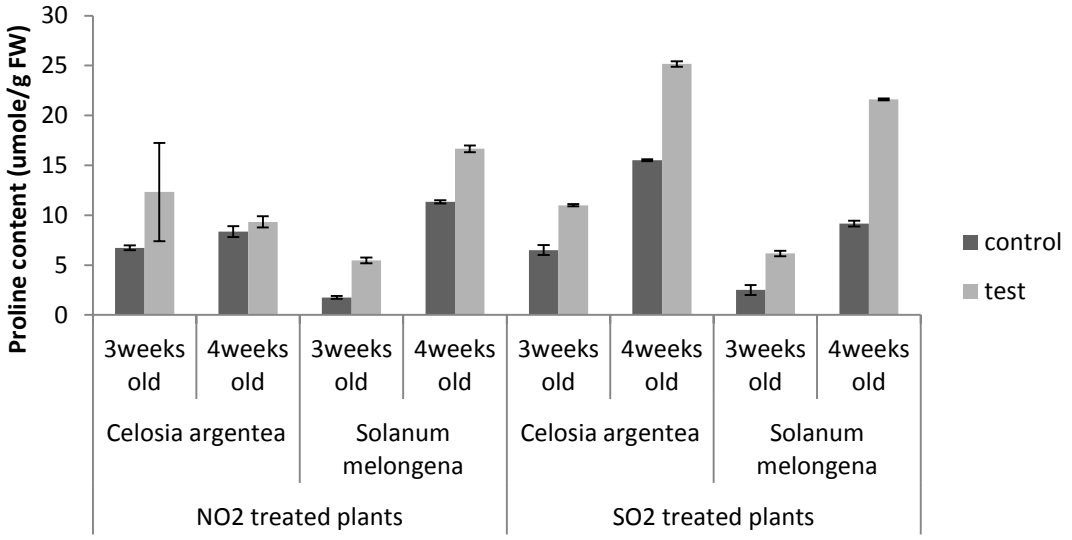

Figure 7: Proline content of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber.

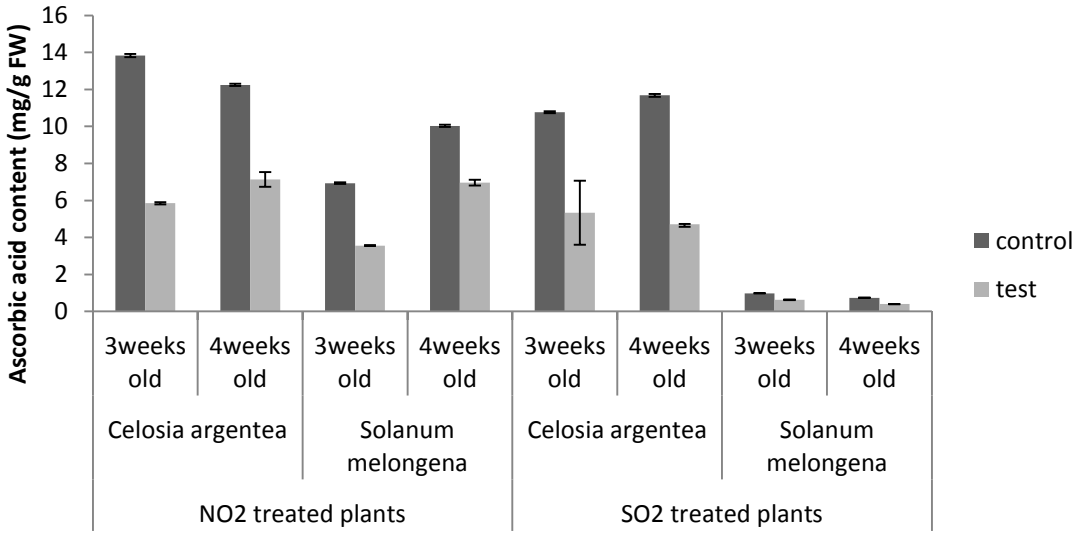

Figure 8: Ascorbic acid of leaf samples harvested from *Celosia argentea* and *Solanum melongena* plants exposed to NO_2 and SO_2 gas in a chamber.

Discussion

Sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) as air pollutants can directly affect plants via leaves or indirectly via soil acidification. When exposed to airborne pollutants such as NO_2 and SO_2, most plants experience physiological changes before exhibiting visible damage to leaves (33). Plants protect themselves against oxidative stress with antioxidant enzymes such as superoxide dismutase, catalase and peroxidase (34). The result of this research shows a significant reduction in chlorophyll a, b and total chlorophyll contents when exposed to NO_2 and SO_2 gases. Peiser and Yang (35) stated that the exposure of the test plants to NO_2 and SO_2 gases results in tissue damage and the release of stress ethylene from photosynthetic and non photosynthetic tissues. NO_2 and SO_2 gases present in the tissues of these plants causes a shift in the cytoplasmic pH. Proton concentration of the cytoplasm is one of the most important factors regulating cellulase activity. SO_2 gas in the cells of plants causes an appreciable acidification of the cytoplasm, this then reacts with water to form sulphurous acid (H_2SO_3) which may then be converted to sulphuric acid (H_2SO_4) (36, 37).

The gas treatments resulted in significant decreases in SOD activity when compared to the control in the *C. argentea* and *S. melongena* leaves after treatment. Similar results were observed by Lijun *et al*. (38) who reported inhibition of SOD by high concentrations of cadmium. In *Arabidopsis thaliana* the total SOD activity decreased three days after methyl jasmonate (JAME) treatment (39). Catalase is the most efficient antioxidant enzyme which protects plant by scavenging free radicals and H_2O_2 (40). Decrease catalase activity in the VTC 1

mutants of *Arabidopsis thaliana* during the course of the UV-B exposure experiment was observed and it could be due to destruction of the peroxisome via rampant lipid peroxidation (41). Furthermore, in the present study, catalase activity was reduced. Catalase reduction could be related to an accumulation of H_2O_2 in the peroxisomes as a strategy to preserve it from total degradation. Thus H_2O_2 could be used as a defense signalizing molecule in other parts of the plant (42). Other reports have registered the decrease in catalase activity as in nodules of *Phaseolus vulgaries* roots under saline stress (43). Chamnongpol *et al*. (44) reported catalase as a mediator of defense response because the reduction in its activity was related to the accumulation of H_2O_2 in tobacco. The catalase activity reduction was also recorded in *Catharanthus roseus* cultures, induced by fungi (45) and by water stress in strawberry (46).Ascorbic acid is known for its function as a powerful antioxidant and for its role in photosynthesis and photoprotection against oxidative stress, including SO_2 (47). Studies indicate that oxidative stress, evoked by atmospheric pollutants enhances the quantity of low molecular antioxidants such as ascorbic acid in cells. Ascorbic acid is very important primary antioxidant which reacts with hydroxyl radical, superoxide and singlet oxygen as well as secondary antioxidant reducing the oxidized form of α-tocopherol (48). Ascorbic acid is known to provide stability to the plant cell membrane during pollution stress and scavenges cytotoxic free radicals, which can otherwise cause lipid peroxidation and destruction of membranes (49). In this study, ascorbic acid level in the test leaves of *C. argentea* and *S. melongena* decreases when compared to the control. It has been reported that resistant species showed increase in ascorbic acid content (49). Important and widespread air pollutants such as SO_2 and NO_2 can produce an oxidative stress in plants, affects physiological process and can cause morphological damages as shown by this study.

Environmental stress conditions generate different responses in higher plants at multiple levels. One common response to abiotic stress (especially drought) conditions which is reported in many studies is the free proline accumulation in leaf tissues of higher plants. Proline has been demonstrated to play osmoprotective functions in protoplasm when plants are under adverse conditions (50). The accumulation of proline in cells, tissues and organs is suggested to function as compatible osmolyte, free radical scavenger, cell redox balancer, potential inhibitor of programmed cell death (PCD), cytosolic pH buffer and stabilizer for subcellular structures during stress (51, 52, 53). The gaseous exposure of studied plants recorded higher values of proline contents in test plants as compared to control. This high accumulation of proline may be attempts by the test plants to tolerate and acclimatise to the stress conditions. The proline content of the test plants was significantly ($\alpha = 0.05$) increased unlike other stress enzymes when compared with the control. New insights on the role of proline in plants were thrown up following expansion of oxidative stress researches. It is clear that proline is multifunctional amino acid with many metabolic pathways. Proline accumulation under stress occurs because of an increase in the amount of P5CS (pyrroline-5-carboxylase synthetase) and a decrease of the activity of proline dehydrogenase (PDH). The two-step oxidation of proline in all eukaryotes is performed at inner mitochondrial membrane by consecutive action of PDH that produces pyrroline-5-carboxylate (P5C) and P5C dehydrogenase (P5CDH) that oxidizes P5C to glutamate. This catabolic route is downregulated in plants during osmotic stress resulting in free proline accumulation (54).

The study has shown that *C. argentea* and *S. melongena* plants exposed to SO_2 and NO_2 gases separately could not recover from the stress after two-day acute trial. Significant reductions in photosynthetic capacity and antioxidant activities (SOD, CAT, POX and ascorbic acid) were recorded. Proline accumulations in the test plants were insufficient to scavenge reactive oxygen species (ROS) generated by the stress from gas exposure. The morphological features observed support this opinion.

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