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# Impacts of simulated acid rain on selected physiological parameters and nutrient value of *Celosia argentea* L.

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**Abstract**
Pot experiment was conducted to assess the effects of Simulated Acid Rain (SAR) on *Celosia argentea*. Seedlings were raised from seeds and 3 weeks old plants of relatively equal height (12 cm) were transplanted, one plant per pot, into plastic pots containing loamy soil. A total of 36 pots were grouped into 2 to represent treatment and control. Plants were allowed to acclimatize for 1 week before treatment was applied. Seedlings were hand sprayed foliarly with 100 ml SAR ($H_2SO_4$ solution) at pH 4.5 and a control level of pH 6.8 every 2 days for 3 weeks. It was observed that SAR significantly ($p < 0.05$) reduced whole plant biomass; relative water content (RWC), chlorophyll, mineral nutrients, carbohydrate and protein contents, but increased the malondialdehyde (MDA) concentration. It was revealed that SAR induced oxidative stress and adversely affected the nutrient value of *C. argentea*.

***Keywords:*** Simulated Acid Rain, *Celosia argentea*, Air pollution, Environment, Nutrition.

## Introduction

The equilibrium pH of normal rain is approximately 5.6 on account of the dissolution of atmospheric $CO_2$. Precipitation having a pH value less than 5.6 is defined as acid rain, while the mean pH of the majority of acidic precipitations is usually 4-5 (Chen *et a*l., 2012). The global increase in industrial activities has lead to elevated level of emission of oxides of nitrogen and sulphur which further produce $HNO_3$ and $H_2SO_4$ respectively after complex photochemical reactions in the atmosphere and subsequently precipitate as acid rain (Chen *et al*., 2013).

Rain with pH as low as 3.0 – 3.5 has been documented in the past (Likens and Bormann, 1974). The sources of these oxides have been linked to automobiles, industrial, coal fired power stations, and smelters (Singh and Agrawal, 2008). These oxides interact with reactants present in the atmosphere in several chemical steps resulting in acid deposition.
Acid rain causes damage to infrastructures and aesthetic materials; it reduces soil fertility through leaching of mineral nutrients; induces toxic metals contamination of underground water; and also destroys aquatic life and vegetation (Singh and Agrawal, 2008).

Plant species exhibit different degrees of susceptibility to the effects of acid rain. Most past studies on the effects of acid rain on higher plants were on the morphological and anatomical

changes, while physiological data are almost absent (Kovacik *et al*., 2011). In Nigeria, economic growth and development is gradually on the increase with establishments of factories and industries in some of the commercial cities by foreign investors. Also, there has been unprecedented increase in the number of imported second hand automobiles into the country. These developments will bring about elevated level of emissions which may result in acid precipitation in the near future. This might affect important crop plants including vegetable such as *Celosia argentea.* Presently, there is paucity of information in Nigeria on local assessment of some of the physiological and nutritional effects of acid rain on crop plants. Therefore, we document the impacts of simulated acid rain on selected physiological parameters and nutrient value of *C. argentea*, a popular leafy vegetable widely cultivated and consumed as source of minerals and vitamins in most parts of Nigeria.

## Materials and methods

*Plant growth and treatment*

Seeds of *C. argentea* were purchased from a local market at Idi Araba, Lagos State, Nigeria in a single batch and enough for the study. Seeds were broadcasted on a nursery bed (1.6 m x 2.4 m) in the Botanical garden of the University of Lagos. After germination, seedlings were nursed for 3 weeks and those with relatively equal height (12 cm) were transplanted, one seedling per pot into 36 plastic pots filled with loamy soil. Plants were grouped into two categories, representing SAR treatment ($H_2SO_4$ solution) and control series and replicated 18 times. Plants were allowed to acclimatize for one week before treatment was applied. Seedlings were hand sprayed foliarly with 100 ml SAR at pH 4.5 and a control level of pH 6.8 every 2 days for 3 weeks. At the end of the treatment period, plants were harvested for analyses.

*Dry weight determination*

Plants were uprooted carefully and washed thoroughly in a running tap water to remove soil particles. After rinsing with distilled water, they were placed in labelled paper bags and oven dried at 65 $^{o}$C for 72 h. The dried samples were weighed using a digital top loading weighing balance (Mettler AE 100) to determine the dry weight.

*Relative water content of leaves*

The fourth leaves from the apices were harvested for the determination of relative water content (RWC). The RWC of each leaf was determined according to the method of Turner (1981) by using the formula RWC (%) = [(fresh weight - dry weight) / (turgid weight – dry weight)] x 100.

*Determination of total chlorophyll*

Plant leaves (0.5g) were ground in 10ml 80% acetone in the dark. After centrifugation at 4000 g for 5 min, the absorbance of the supernatant was read at 645 nm and 663 nm (Arnon, 1949). The total chlorophyll content was calculated using the formula given by Machlachlan and Zalik (1963).

*Lipid peroxidation assay*

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang and Jin (2005). Fresh leaves (0.5 g) were

homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 g for 5 min. The supernatant (1 ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 minutes in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as $6.45(A_{532}-A_{600})-0.56\ A_{450}$ (Wang and Jin, 2005).

*Mineral nutrients quantification*

The quantification of mineral nutrients (K, P, Na, Ca, Mg, Fe Zn and Cu) was done according to the method described by Kovacik *et al.* (2009). Dried leaves (0.5 g) were ground to a smooth powder and soaked overnight in a mixture of $HNO_3$ and $H_2O_2$ (10 ml + 10 ml) at room temperature. It was evaporated to dryness the next day at 90 $^oC$ in a water bath and the residue was dissolved in 5% $HNO_3$. Measurement was done using atomic absorption spectrophotometer and air-acetylene flame.

*Carbohydrate determination*

Carbohydrate level in the shoot of the dried plant samples was determined using anthrone-$H_2SO_4$ reagent as described by Hansen and Moller (1975). The glucose content was multiplied by 0.9 to obtain the amount of starch (Nakano *et al*., 2000).

Protein determination

The amount of protein was determined using the modified Kjedahl method for the estimation of total organic nitrogen in the dried leaf samples as described by Eastin (1978). The nitrogen value in the sample was multiplied by 6.25, to obtain the amount of protein (Ramalho *et al.,* 2000).

*Statistical analysis*

Means of 3 replicates as well as the standard error (SE) were determined. The test of significance between the treatments was done using a two-sample t-test.

**Results**

Data showing the effect of acid rain on the biomass accumulation in *C. argentea* is depicted in Figure 1. It was observed that SAR significantly reduced the biomass production. While the control plants had a mean value of 6.1 ± 0.1g, plants treated with simulated acid rain had a mean value of 4.6 ± 0.3 g.

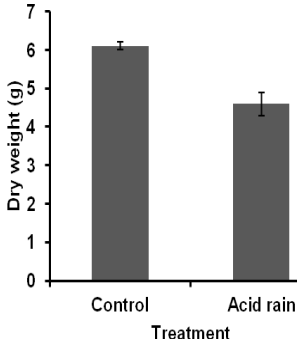


**Figure 1: Whole plant dry weight of *C. argentea* exposed to simulated acid rain.**
*{Error bars represent standard error (n=3)}*

The effect of acid rain treatment on the relative water content (RWC) of *C. argentea* leaves is as shown in figure 2. It was noticed that exposure to acid rain had significant ($p < 0.05$) reduction in the RWC of the plant. After 3 weeks of treatment, the RWC of the control had a mean value of 76.8 % while plants treated with acid rain had 64.4 %.

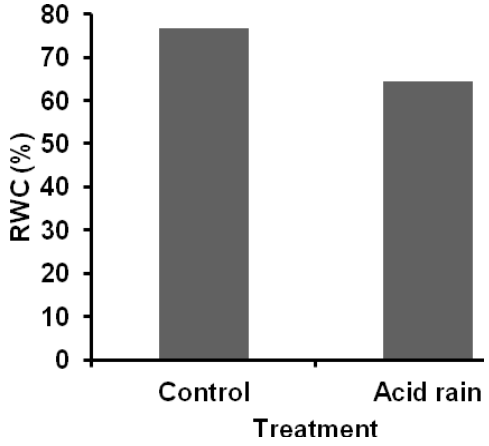


**Figure 2: Relative water content of *C. argentea* leaves treated with acid rain.**

It was observed in this study that simulated acid rain significantly reduced the total chlorophyll content of *C. argentea* (Figure 3). From the data, the control plants had a mean value of 1.05 ± 0.03 mg $g^{-1}$ f. wt as against 0.73 ± 0.04 mg $g^{-1}$ f wt extracted from plants subjected to acid rain.

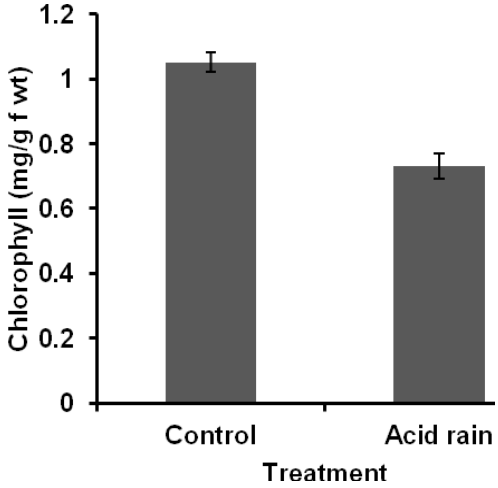


**Figure 3: Total chlorophyll content of *C. argentea* exposed to acid rain.**
**{***Error bars represent standard error (n=3)}*

Figure 4 shows the level of lipid peroxidation in the leaves of treated and untreated *C. argentea*. To evaluate this parameter, malondialdehyde (MDA) content, an end product of poly unsaturated fatty acids peroxidation was measured. It was observed that SAR significantly increased the MDA content in the plant. The control had a mean value of 0.83 ± 0.03 mg $g^{-1}$ f wt as against

that of the treated plants which a mean value of 1.26 ± 0.01 mg $g^{-1}$ f wt. The selected mineral nutrients present in *C. argentea* indicated that SAR significantly reduced the concentration of potassium, phosphorus, calcium and magnesium; while the amounts of sodium, iron and copper were not significantly affected (Table 1). The data showed that only macronutrients accumulation was significantly affected. Figure 5 shows the effect of acid rain on the carbohydrate content of *C. argentea.* It was observed in this study that SAR significantly decreased the carbohydrate content in the treated plants. The carbohydrate content of the control was significantly ($p < 0.05$) higher than the values observed for plants exposed to SAR. The control had a mean value of 43.31 ± 2.87 mg/ g dry wt, compared to 31.87 ± 3.18 mg/ g dry wt observed for plants treated with SAR.

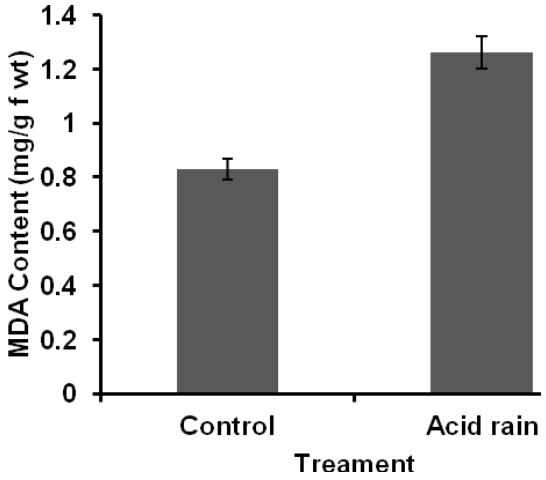


**Figure 4: Malondialdehyde content in *C. argentea* leaves as affected by acid rain.**
*{Error bars represent standard error (n=3)}*

**Table 1: Selected mineral nutrients (mg/g dry wt) in *C. argentea* leaves exposed to acid rain**

| Elements | K | P | Na | Ca | Mg | Fe | Cu |
|---|---|---|---|---|---|---|---|
| Control | 7.18±0.11 | 5.34±0.21 | 4.31±0.15 | 4.42±0.35 | 5.78±0.26 | 0.53±0.80 | 0.038±0.015 |
| Acid rain | 5.71±0.16* | 3.14±0.15* | 3.98±0.26 | 3.32±0.16* | 2.63±0.21* | 0.48±0.11 | 0.032±0.023 |

*Data are means ± standard errors (n=3). Treatment values followed by * are significantly lower than the control according to two-sample t-test at ($p < 0.05$).*

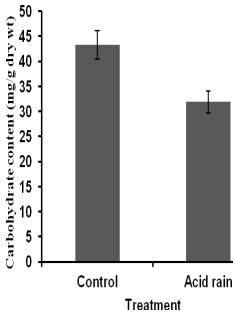


**Figure 5: Carbohydrate content of *C. argentea* as affected by simulated acid rain.**
*{Error bars represent standard error (n=3)}*

Data showing the effect of SAR on the protein content of *C. argentea* leaves is depicted in Figure 6. The result showed that SAR treatment significantly reduced the protein content of the plant. It was observed in this study that the control plants had a mean value of 8.12 ± 0.60 mg $g^{-1}$ dry wt as against that of the treated with a mean value of 5.27 ± 0.4 mg $g^{-1}$ dry wt.

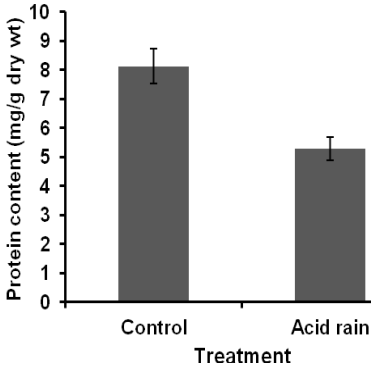


**Figure 6**: Protein content of *C. argentea* exposed to simulated acid rain.
*{Error bars represent standard error (n=3)}.*

**Discussion**

This research was conducted to assess some of the physiological responses of *C. argentea* to SAR and nutritional content of SAR treated and control plants. Data from this study showed that SAR significantly inhibited growth in *C. argentea* confirming the sensitivity of this species to the tested pH 4.5. The reduction in whole plant dry weight could be attributed to acid rain effect on photosynthesis via the disruption of enzymes activities and decreased pigment concentration. The result conformed to the findings reported by other authors (Irving, 1983; Khan and Devpura, 2011).

The reduction in RWC of the acid stressed plants could be related to cuticle damage that would result in increased water loss via transpiration from the leaves. Acid rain could also affect the integrity of the membrane by destroying the lipid bi-layer thereby causing cell membrane leakage.

A significant reduction in the level of total chlorophyll was observed in the leaves of plants grown under acid stress condition. Reduction in chlorophyll content of plant exposed to SAR has been observed in different plant species (Evans, 1984; Haslam *et al.,* 2003; Khan and Devpura, 2004; Kovacik *et al*., 2011). The reason could be that acid rain adversely affected the biosynthesis of the pigment by inhibiting enzymes activities and the removal of magnesium ion from the tetrapyrol ring of the chlorophyll molecules by hydrogen ion (Foster, 1990).

A common aspect of most adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several sub cellular compartments of the plant cell (Van Breusegem, *et al*., 2001). Reactive oxygen species can occur as by-products of regular cellular metabolism such as in photosynthesis. However, under stress conditions, their formation is usually exacerbated. It was observed in this study that simulated acid rain caused significant increase in ROS generation. This result agreed with the findings reported by Gabara, *et al*.,

2003; Wyrwicka and Sklodowska, 2006; Kovacik, *et al*., 2011). Our study showed that macronutrients were more depleted by SAR in comparison with micronutrients. The marked decrease in the mineral elements in acid-treated plants could be ascribed to increased solubility of the elements and elution from damaged cells. Similar result has also been reported by Ferenbaugh (1975).

The significant reduction in carbohydrate content in acid-treated plants could be attributed to photosynthetic impairment due to low chlorophyll and mineral nutrients contents that are essential for photosynthetic carbon fixation efficiency. This result agreed with the findings reported by Khan and Devpura (2004).

There was a significant reduction in protein content of the treated plants compared to the control. The result agreed with the findings earlier reported by Kovacik *et al.* (2011) that acid rain treatment on epiphytic plants resulted in decreased protein content. The decrease in protein content in the treated plants could be related to a remarkable inhibition in glutamine synthetase (GS) and glutamate synthase (GOGAT) activities (Nemat Alla *et al.,* 2008). The failure in GS-GOGAT system function would cause accumulation of unassimilated ammonia and consequently, could lead to diminution in amino acid formation with a subsequent drop in protein synthesis.

**Conclusion**

In conclusion, data from this study showed that SAR caused a significant decrease in whole plant biomass, RWC, chlorophyll, mineral nutrients, carbohydrate and protein contents, while converse was true for malondialdehyde (MDA) content. The data presented in this work underscored the adverse effects of acid rain on the growth, yield and nutrient value of *C. argentea.*

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