

## Effect of simulated nitric and sulphuric acid rains on the nutrient content of *Amaranthus hybridus*

L.

### Abstract:

**Aims:** To evaluate the potential effects of simulated nitric and sulphuric acid rain on the proximate, minerals, vitamins and antinutrients contents of *Amaranthus hybridus* Linnaeus (*A. hybridus*). **Study design:** The study was carried out in a complete randomized block design. **Place and duration:** Department of Botany, University of Calabar, Calabar, Nigeria between March and June, 2016. **Methodology:** Plants were harvested at two months post simulated nitric and sulphuric acid rain treatments. The leaf, stem and root were separated, dried, milled into powder and used for proximate, minerals, vitamins and antinutrients analysis according to standard methods. Simulated acid rain pH 2.0, pH 3.0, pH 4.0 and control pH 6.0 were used. **Results:** Results revealed significant ( $P = .05$ ) decrease in proximate, minerals or elements, vitamins and antinutrients of *A. hybridus* treated with simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) when compared with the control. Reductions induced by acids rain were significant according to pH of acid concentrations. Nutrients composition in the plant parts varied with more protein, carbohydrate, moisture, K, Ca, Fe, and Cu in leaf. Stem part had more of ash, fat, fibre, Zn, vitamin C, hydrocyanic acid. Others had similar amounts in leaf, stem and root. Highest nutrient reductions induced by SNAR and SSAR occurred at pH 2.0 and lowest reductions at pH 4.0, pH 6.0 had the highest amount of nutrients. Reduction in minerals imposed by acid rain on iron in leaf at pH 2.0 were  $6.30 \pm 0.01$  (SNAR),  $6.30 \pm 0.01$  (SSAR) compared with control pH 6.0 value of  $14.03 \pm 0.01$  mg/100 g, the stem had values of  $5.01 \pm 0.01$  (SNAR),  $5.65 \pm 0.01$  (SSAR) compared to  $14.09 \pm 0.01$  mg/100 g value for the control pH 6.0, reduction values for the root were  $4.28 \pm 0.01$  (SNAR),  $4.28 \pm 0.01$  (SSAR) when compared with control pH 6.0 value of  $14.88 \pm 0.01$  mg/100 g. Decrease in protein values caused by acid rain at pH 2.0, 3.0 and 4.0 had values of  $12.69 \pm 0.01$ ,  $12.97 \pm 0.03$ ,  $13.76 \pm 0.03$  for SNAR and  $9.39 \pm 0.01$ ,  $10.82 \pm 0.02$ ,  $11.82 \pm 0.06$  g/100 g for SSAR respectively in leaf. **Conclusion:** Exposure of *Amaranthus hybridus* to simulated nitric and sulphuric acid rains adversely altered its nutrient content.

**Keywords:** *Amaranthus hybridus*, Simulated nitric and sulphuric acid rain, proximate, minerals, vitamins, antinutrients.

### 1. Introduction

In the last few decades, air pollution caused by a number of factors including burning of fossil fuel, aerosols, air conditioners, and natural environmental changes such as volcanic activities has become a problem. The quality of the growing environment of the plant is of fundamental importance to its survival, growth and productivity. Industrial activities accompanied by air pollution have become a major source of acid rain posing a threat to healthy existence of natural and cultivated crops [1], [2]. Large amounts of

gases (sulphur oxides and nitrogen oxides) emitted into the atmosphere from industrial plant and other sources cause profound deterioration of urban air quality [3]. These gases undergo series of chemical reactions; sulphur dioxide ( $\text{SO}_2$ ) react with oxygen in the air to form sulphur trioxide ( $\text{SO}_3$ ), which in-turn reacts rapidly with atmospheric moisture ( $\text{H}_2\text{O}$ ) to form sulphuric acid ( $\text{H}_2\text{SO}_4$ ) [4]. Similarly oxides of nitrogen react with moisture content in the air to produce nitric acid ( $\text{HNO}_3$ ) which falls as acid rain. Under natural conditions, atmospheric precipitation is slightly acidic due to the dissolution of carbon dioxide in the atmosphere [5]. Rain is considered acidic when the concentration of  $\text{H}^+$  ions is greater than  $2.5\mu\text{eq}^{-1}$  and pH values lower than 5.6. Acid deposition may cause a decline in plant health and growth [6].

*Amaranthus hybridus* is a vegetable that serve as an indispensable constituent in diet supplying the body with minerals, vitamins, proteins, fibre, hormones, microelements and broadens the food base of the people. It is a crop that plays an important role in traditional medical practice and local diet; improve household income generation. Nutritional composition of this vegetable has been reported to possess high amount of protein, fat, fibre, ash, mineral elements, vitamins and amino acids [7]. It is widely cultivated on a commercial scale in southern Nigeria where they are cooked with yam and used in soup preparation. Urban or homestead (vegetable) farming is now an important phenomenon in major towns in Nigeria because of knowledge of phytonutrients. Nigeria and other third world countries depend on starch-based foods and the poor populace cannot afford animal and other forms of supplementary nutrients, hence the need to feed on vegetables to meet the body's nutritional requirement [8].

The susceptibility of plants to acid rain has been reported to vary greatly with plant species. Reductions in plant nutrients in respect of acid rain have been studied; decrease in vitamins A, E and C content of strawberry plants sprayed with simulated acid rain [9]. [10] reported a reduction in N concentration in leaves of apple by rains of low pH but observed no significant difference in levels of  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ . [11] reported a reduction Mg, Ca, Fe, K, and N content of *Solanum lycopersicum* at low pH.

Information on acid rain effect on the nutrient content of plants is scare. The determination of the nutrient contents of plants, which are natural sources and the effect of acid rain which is the result of environmental pollution on nutrient levels are important. This is needful for future plans and for the measures to be taken. Owing to population increase and anthropogenic activities acid rain is on the

increase in Nigeria. In view of the importance of this vegetable to the Nigerian people and the adverse effect of acid rain, the present study is aimed at determining the effect of simulated nitric (SNAR) and sulphuric acid rain (SSAR) on the nutrient content of *Amaranthus hybridus* L.

## **2. Materials and Methods**

### **2.1 Seeds and materials collection**

Seeds of *A. hybridus* were obtained from a farmer in Calabar South Local Government Area in Calabar. Polyethylene bags of 16 mm in diameter used for planting were obtained from the Ministry of Agriculture, Calabar. Nitric and sulphuric acids were purchased from a Scientific Store all in Calabar, Nigeria.

### **2.2 Seeds planting**

The experiment was conducted in a greenhouse at University of Calabar, Calabar, Nigeria (latitude 4.952'N and longitude 8.341'E) at  $25\pm3^{\circ}\text{C}$ . *Amaranthus hybridus* plants were grown in a greenhouse to minimize pest infestation and uniformity of sunlight and water supply. Seeds were planted and the young seedlings grown in a nursery for weeks before transplanted into planting bags. The transplanted seedlings were watered every two days for two weeks before the application of the simulated acid rain treatment. A total of thirty five bags were used, fifteen bags each for nitric acid and sulphuric acid rain and five bags for the control. Each pH treatment had five replicates and the poly bags arranged in a complete randomized block design.

### **2.3 Preparation of simulated acid rain**

Simulated nitric acid (SNAR) and sulphuric acid rain (SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately prepared using 30 ml of each of the acids for pH 2.0, 20.1 ml for pH 3.0 and 10.2 ml for pH 4.0 and a control pH of 6.0 using a Deluxe pH meter and distilled water (Mofunanya's method). The different simulated acid rain concentrations were sprayed using a domestic hand-spraying unit on the plants as well as soil by the method of [12]. Fifty (50 ml) of each acid solution was applied at the initial stage every two days interval for thirteen weeks. The amount increased in the course of plant growth.

### **2.4 Samples preparation**

At the end of thirteen months post simulated acid rain treatment, the whole plants were harvested and the parts (Leaf, Stem and Root) separated and sun-dried for one week. The different parts were milled separately into powdered form in an electric mill (National Food Grinder, Model MK 308, Japan). The pulverized samples were used to analyze the potential effect of simulated nitric and sulphuric acid rains on the proximate, minerals, vitamins and antinutrients contents of *Amaranthus hybridus*.

## 2.5 Samples analysis

Standard methods of analysis were employed. Crude protein was analyzed by the Kjeldahl method. Ash, fat and fibre content by the method of AOAC 2006 [13]. Minerals present in plant parts of acid treated and control plants of *A. hybridus*; Calcium, magnesium, iron, zinc and copper were analyzed using the atomic absorption spectrophotometer. Sodium and potassium were estimated by flame photometry, phosphorus by colorimetric method as outlined by the method of AOAC 2006 [13]. Antinutrients and Vitamins A, B, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, E and C by methods of [14].

## 2.6 Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) at  $P = .05$  using the Statistical Package for Social Science, Version 15.0 [15].

## Results

### 3.1 Effect of simulated nitric acid rain and sulphuric acid rain on proximate composition of plant organs of *Amaranthus hybridus* L

Data analysis of effect of simulated nitric acid rain and sulphuric acid rain on all proximate (ash, protein, fat, fibre, carbohydrates and moisture) nutrients of *Amaranthus hybridus* plant organs or parts (leaf, stem and root) analyzed revealed reduction in content. The quantities of these nutrients varied in plant parts. Ash, fat and fibre (proximate) content were more in stem than in leaf and root samples. Protein, carbohydrate and moisture content were more in leaf than in stem and root samples (Table 1). Ash, fat and fibre contents in leaf and root did not differ. Significant ( $P = .05$ ) mean values reduction for fibre content in stem, leaf and root at pH 2.0 (SNAR) were  $6.47 \pm 0.01$ ,  $3.32 \pm 0.01$  and  $3.32 \pm 0.01$  g/100 g, while

113 SSAR had values of  $6.42 \pm 0.01$ ,  $3.22 \pm 0.01$  and  $3.52 \pm 0.01$  g/100 g compared with control pH 6.0 values of  
 114  $8.68 \pm 0.01$ ,  $7.81 \pm 0.01$  and  $7.90 \pm 0.01$  g/100 g respectively. Fibre content in SSAR treated leaf sample did  
 115 not differ at pH 3.0 and pH 4.0. The control pH 6.0 had highest amounts of proximate nutrients when  
 116 compared with simulated acid treated samples. Highest nutrient reductions in plant parts of *A. hybridus*  
 117 occurred at pH 2.0. The leaf sample had more protein content followed by the stem and root. Leaf protein  
 118 content of SNAR treated plant organs had mean values reduction of  $12.69 \pm 0.01$ ,  $12.97 \pm 0.03$ ,  $13.76 \pm 0.03$   
 119 g/100 g at pH 2.0, 3.0 and 4.0 and values of  $9.39 \pm 0.01$ ,  $10.82 \pm 0.02$  and  $11.82 \pm 0.06$  g/100 g for SSAR  
 120 treated plant respectively compared with control pH 6.0 value of  $17.80 \pm 0.01$  g/100 g. The stem had mean  
 121 protein reduction values of  $11.39 \pm 0.03$  (pH 2.0),  $12.52 \pm 0.01$  (pH 3.0) and  $13.62 \pm 0.01$  g/100 g (pH 6.0) for  
 122 SNAR,  $10.26 \pm 0.01$  (pH 2.0),  $10.48 \pm 0.03$  (pH 3.0) and  $10.50 \pm 0.01$  g/100 g (pH 4.0) for SSAR compared  
 123 with control value of  $17.60 \pm 0.02$  g/100 g (pH 6.0). Root protein had significant ( $P = .05$ ) mean reduction  
 124 values of  $10.68 \pm 0.01$  (pH 2.0),  $11.72 \pm 0.01$  (pH 3.0) and  $12.86 \pm 0.02$  (pH 4.0) for SNAR,  $11.27 \pm 0.01$  (pH  
 125 2.0),  $11.42 \pm 0.01$  (pH 3.0) and  $12.43 \pm 0.01$  g/100 g (pH 4.0) as against control (pH 6.0) value of  
 126  $17.49 \pm 0.01$  g/100 g. There were significant reductions in protein and moisture at each pH concentration  
 127 compared to the control Table 1.

128

129 **Table 1: Effect of simulated nitric acid rain and sulphuric acid rain on proximate composition of**  
 130 **plant parts of *Amaranthus hybridus* L.**

| Proximate nutrients | g/100 g                      |             |            |                                            |            |            |               |
|---------------------|------------------------------|-------------|------------|--------------------------------------------|------------|------------|---------------|
|                     | HNO <sub>3</sub> (pH Concs.) |             |            | H <sub>2</sub> SO <sub>4</sub> (pH Concs.) |            |            |               |
|                     | 2.0                          | 3.0         | 4.0        | 2.0                                        | 3.0        | 4.0        | Control (6.0) |
| <b>Ash</b>          |                              |             |            |                                            |            |            |               |
| Leaf                | 10.86±0.01                   | 11.01±0.01  | 11.01±0.01 | 10.89±0.01                                 | 11.72±0.01 | 11.72±0.01 | 13.90±0.01    |
| Stem                | 11.29±0.01                   | 11.27±0.02  | 11.37±0.02 | 11.96±0.01                                 | 12.02±0.01 | 12.44±0.01 | 14.00±0.01    |
| Root                | 10.96±0.01                   | 11.62±0.01  | 11.77±0.01 | 11.59±0.01                                 | 11.82±0.02 | 11.90±0.01 | 13.96±0.01    |
| <b>Protein</b>      |                              |             |            |                                            |            |            |               |
| Leaf                | 12.69±0.01                   | 12.97± 0.03 | 13.76±0.03 | 9.39± 0.01                                 | 10.82±0.02 | 11.82±0.06 | 18.80±0.01    |
| Stem                | 11.39±0.03                   | 12.52±0.01  | 13.62±0.01 | 10.26±0.01                                 | 10.48±0.03 | 10.50±0.01 | 17.60±0.02    |
| Root                | 10.68±0.01                   | 11.72±0.01  | 12.86±0.02 | 11.27±0.01                                 | 11.42±0.01 | 12.43±0.01 | 17.49±0.01    |
| <b>Fat</b>          |                              |             |            |                                            |            |            |               |

|                     |            |            |            |            |            |            |            |
|---------------------|------------|------------|------------|------------|------------|------------|------------|
| Leaf                | 3.70±0.01  | 3.86±0.01  | 3.88±0.03  | 3.80±0.01  | 4.50±0.01  | 4.65±0.02  | 4.70±0.01  |
| Stem                | 4.06±0.01  | 4.37±0.01  | 4.45±0.03  | 4.12±0.02  | 4.49±0.01  | 4.31±0.01  | 4.40±0.03  |
| Root                | 3.76±0.01  | 3.82±0.01  | 4.22±0.01  | 4.35±0.01  | 4.38±0.01  | 4.69±0.01  | 4.78±0.01  |
| <b>Fibre</b>        |            |            |            |            |            |            |            |
| Leaf                | 3.32±0.01  | 4.72±0.01  | 4.97±0.01  | 3.22±0.01  | 4.78±0.01  | 4.78±0.01  | 7.81±0.01  |
| Stem                | 6.47±0.01  | 6.82±0.01  | 6.96±0.01  | 6.42±0.01  | 6.51±0.01  | 6.70±0.01  | 8.68±0.01  |
| Root                | 3.32±0.01  | 3.76±0.01  | 4.52±0.01  | 3.52±0.01  | 6.62±0.01  | 3.71±0.01  | 7.90±0.01  |
| <b>Carbohydrate</b> |            |            |            |            |            |            |            |
| Leaf                | 83.21±0.01 | 83.47±0.01 | 84.45±0.01 | 82.65±0.01 | 84.13±0.01 | 85.13±0.01 | 90.13±0.01 |
| Stem                | 80.15±0.01 | 80.40±0.01 | 80.41±0.01 | 80.14±0.01 | 82.04±0.01 | 83.01±0.01 | 90.87±0.01 |
| Root                | 82.21±0.01 | 83.14±0.01 | 83.20±0.01 | 83.48±0.01 | 84.14±0.01 | 83.51±0.01 | 90.49±0.01 |
| <b>Moisture</b>     |            |            |            |            |            |            |            |
| Leaf                | 79.02±0.01 | 80.10±0.01 | 83.71±0.01 | 78.99±0.01 | 80.25±0.01 | 84.10±0.01 | 88.02±0.01 |
| Stem                | 77.03±0.01 | 78.11±0.01 | 83.12±0.01 | 77.10±0.01 | 79.21±0.01 | 83.20±0.01 | 86.14±0.01 |
| Root                | 78.51±0.01 | 79.18±0.01 | 82.43±0.01 | 78.52±0.01 | 79.20±0.01 | 82.23±0.01 | 84.57±0.01 |

131 • Means of three replicates  $\pm$  SEM, n =3,  $P = .05$ .

132 •  $\text{HNO}_3$  = Nitric acid,  $\text{H}_2\text{SO}_4$  = Sulphuric acid, Conc. = Concentration

133 • SNAR = Simulated nitric acid rain, SSAR = Simulated sulphuric acid rain

## 134 3.2 Effect of simulated nitric acid rain and sulphuric acid rain on mineral composition of

## 135 plant parts of *Amaranthus hybridus* L.

136 Analysis of variance on results of effects of SNAR and SSAR on mineral content of *A. hybridus* revealed

137 significant ( $P = .05$ ) reductions in K, Mg, Ca, Na, Fe, Mn. The content of copper in acid rains treated and

138 control samples did not differ at pH 2.0 and 3.0 but was significantly higher at pH 4.0. Reduction in

139 minerals caused by SNAR and SSAR varied according to pH concentrations with highest mineral

140 nutrients reduction obtained at pH 2.0 and lowest at pH 4.0. The pH 6.0 had the highest amounts of

141 mineral nutrients when compared with acid rains treated plant parts. The amounts of minerals possess by

142 each plant part varied. Results revealed significant ( $P = .05$ ) variation in the amount of K, Ca, Fe and Mg

143 present in the plant parts of *A. hybridus*. No variation in quantities of Mg and Fe was obtained in control

144 plant parts (Table 2). Potassium, Na, Ca, Mg and Fe were more in *A. hybridus* leaf than in stem and root.

145 Reductions in mineral nutrients posed by SNAR on K content of leaf, stem and root at pH 2.0 were

146  $20.08 \pm 0.01$ ,  $18.77 \pm 0.01$  and  $17.06 \pm 0.01$  mg/100 g. Corresponding values for SSAR at pH 2.0 were

147  $24.45 \pm 0.01$ ,  $21.43 \pm 0.01$  and  $19.07 \pm 0.01$  mg/100 g as against control pH 6.0 values of  $54.03 \pm 0.01$ ,

148 50.90±0.01 and 43.01±0.01 mg/100 g. Mean reduction values obtained for Ca at pH 2.0 for SNAR were  
 149 leaf (25.30±0.01), stem (24.56±0.01) and root (23.26±0.01), SSAR had 20.37±0.01 (leaf), 19.77±0.01  
 150 (stem) and 19.01±0.01 (root) when compared with mean values of 43.89±0.01 (leaf), 42.73±0.01 (stem)  
 151 and 40.09±0.01 mg/100 g (root) at pH 6.0. Calcium was more in plant parts of SNAR than SSAR.  
 152 Manganese in stem part of SNAR plant at pH 2.0 (0.06±0.01), pH 3.0 (0.07±0.01) and pH 4.0 (0.09±0.01)  
 153 was higher than that in SSAR treated plant part of pH 2.0 (0.04±0.01), pH 3.0 (0.04±0.01) and pH 4.0  
 154 (0.05±0.01) but significantly lower than the control pH 6.0 (3.09±0.01 mg/100 g) (Table 2).

155 **Table 2: Effect of simulated nitric acid rain and sulphuric acid rain on mineral composition of**  
 156 **plant parts of *Amaranthus hybridus* L.**

| Minerals              | mg/100 g                     |             |             |                                            |             |             | Control (6.0) |
|-----------------------|------------------------------|-------------|-------------|--------------------------------------------|-------------|-------------|---------------|
|                       | HNO <sub>3</sub> (pH Concs.) |             |             | H <sub>2</sub> SO <sub>4</sub> (pH Concs.) |             |             |               |
|                       | 2.0                          | 3.0         | 4.0         | 2.0                                        | 3.0         | 4.0         |               |
| <b>Potassium (K)</b>  |                              |             |             |                                            |             |             |               |
| Leaf                  | 20.08±0.01                   | 22.06±0.01  | 26.22±0.01  | 24.45±0.01                                 | 26.52±0.01  | 25.33±0.01  | 54.03±0.01    |
| Stem                  | 18.77±0.01                   | 19.83±0.01  | 24.01±0.01  | 21.43±0.01                                 | 23.55±0.01  | 24.65±0.01  | 50.90±0.01    |
| Root                  | 17.06±0.01                   | 18.30±0.01  | 20.06±0.01  | 19.07±0.01                                 | 21.22±0.01  | 22.22±0.01  | 43.01±0.01    |
| <b>Sodium (Na)</b>    |                              |             |             |                                            |             |             |               |
| Leaf                  | 4.90±0.01                    | 5.15±0.01   | 5.47±0.01   | 5.34±0.01                                  | 5.62±0.01   | 5.66±0.01   | 6.90±0.01     |
| Stem                  | 4.52±0.01                    | 5.22±0.01   | 5.72±0.01   | 3.82±0.01                                  | 3.92±0.01   | 3.90±0.01   | 6.90±0.01     |
| Root                  | 4.38±0.01                    | 4.45±0.01   | 4.66±0.01   | 4.30±0.01                                  | 4.40±0.01   | 4.40±0.01   | 5.09±0.01     |
| <b>Calcium (Ca)</b>   |                              |             |             |                                            |             |             |               |
| Leaf                  | 25.30±0.01                   | 25.39±0.01  | 35.12±0.01  | 20.37±0.01                                 | 20.88±0.01  | 20.89±0.01  | 43.89±0.01    |
| Stem                  | 24.56±0.01                   | 24.32±0.01  | 24.52±0.01  | 19.77±0.01                                 | 20.38±0.01  | 20.42±0.01  | 42.73±0.01    |
| Root                  | 23.26±0.01                   | 23.55±0.01  | 23.60±0.01  | 19.01±0.01                                 | 19.60±0.01  | 19.99±0.01  | 40.09±0.01    |
| <b>Magnesium (Mg)</b> |                              |             |             |                                            |             |             |               |
| Leaf                  | 171.37±0.01                  | 171.39±0.01 | 171.58±0.01 | 172.51±0.01                                | 172.94±0.01 | 172.94±0.01 | 232.84±0.01   |
| Stem                  | 170.42±0.01                  | 170.47±0.01 | 170.47±0.01 | 171.45±0.01                                | 171.52±0.01 | 171.62±0.01 | 232.57±0.01   |
| Root                  | 170.18±0.01                  | 170.25±0.01 | 170.41±0.01 | 170.40±0.01                                | 170.50±0.01 | 170.50±0.01 | 232.09±0.01   |
| <b>Iron (Fe)</b>      |                              |             |             |                                            |             |             |               |
| Leaf                  | 6.30±0.01                    | 6.31±0.01   | 6.30±0.01   | 6.30±0.01                                  | 6.30±0.01   | 6.30±0.01   | 14.03±0.01    |
| Stem                  | 5.01±0.01                    | 5.04±0.01   | 5.04±0.01   | 5.65±0.01                                  | 6.67±0.01   | 6.81±0.01   | 14.09±0.01    |
| Root                  | 4.28±0.01                    | 4.28±0.01   | 4.29±0.01   | 4.28±0.01                                  | 4.28±0.01   | 4.28±0.01   | 14.88±0.01    |
| <b>Zinc (Cu)</b>      |                              |             |             |                                            |             |             |               |
| Leaf                  | 0.03±0.01                    | 0.05±0.01   | 0.05±0.01   | 0.03±0.01                                  | 0.05±0.01   | 0.56±0.01   | 3.40±0.01     |
| Stem                  | 0.04±0.01                    | 0.06±0.01   | 0.07±0.01   | 0.20±0.01                                  | 0.03±0.01   | 0.30±0.01   | 3.48±0.01     |
| Root                  | 0.04±0.01                    | 0.06±0.01   | 0.09±0.01   | 0.06±0.01                                  | 0.09±0.01   | 0.09±0.01   | 4.09±0.01     |
| <b>Copper (Cu)</b>    |                              |             |             |                                            |             |             |               |
| Leaf                  | 0.03±0.01                    | 0.04±0.01   | 0.06±0.01   | 0.02±0.01                                  | 0.03±0.01   | 0.04±0.01   | 0.03±0.01     |
| Stem                  | 0.01±0.01                    | 0.01±0.01   | 0.02±0.01   | 0.04±0.01                                  | 0.04±0.01   | 0.04±0.01   | 0.03±0.01     |

|                       |           |           |           |           |           |           |           |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Root                  | 0.02±0.01 | 0.03±0.01 | 0.03±0.01 | 0.02±0.01 | 0.02±0.01 | 0.09±0.01 | 0.04±0.01 |
| <b>Manganese (Mn)</b> |           |           |           |           |           |           |           |
| Leaf                  | 0.03±0.01 | 0.04±0.01 | 0.05±0.01 | 0.05±0.01 | 0.05±0.01 | 0.07±0.01 | 3.07±0.01 |
| Stem                  | 0.06±0.01 | 0.07±0.01 | 0.09±0.01 | 0.04±0.01 | 0.04±0.01 | 0.05±0.01 | 3.09±0.01 |
| Root                  | 0.04±0.01 | 0.06±0.01 | 0.08±0.01 | 0.08±0.01 | 0.08±0.01 | 0.10±0.01 | 4.09±0.01 |

157 • Means of three replicates ± SEM,  $P = .05$ .

158 •  $\text{HNO}_3$  = Nitric acid,  $\text{H}_2\text{SO}_4$  = Sulphuric acid, Concs. = Concentration

159 • SNAR = Simulated nitric acid rain, SSAR = Simulated sulphuric acid rain

160

### 161 3.3 Effect of simulated nitric and sulphuric acid on vitamin composition of plant parts of

#### 162 *Amaranthus hybridus* L.

163 Data analysis revealed significant ( $P = .05$ ) reductions in all the vitamins content of acid rain treated plant  
164 organs which also varied according to pH concentrations. Vitamins A and C were more in leaf plant part  
165 than in stem and root. Results also revealed a general trend of highest reduction at pH 2.0 and lowest  
166 reduction at pH 4.0 compared with control pH 6.0. Highest reduction is depicted by lower values and  
167 lower reductions shown by higher values. Quantities of vitamins E, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>6</sub> in leaf, stem and root  
168 differed statistically. Significant ( $P = .05$ ) reduction in vitamin C at pH 2.0, 3.0 and 4.0 in SNAR and SSAR  
169 compared with the control pH 6.0 are shown (Table 3). Stem part of *A. hybridus* had the highest amount  
170 of vitamin C. Vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, E and C were significantly reduced in acid rain treated plant  
171 organs or parts. Reductions induced by SNAR on vitamin A content of *A. hybridus* plant organs at pH 2.0  
172 had mean reduction values of 2.20±0.01, 2.18±0.01 and 1.85±0.01 µg/dl for leaf, stem and root, while  
173 SSAR had mean values of 1.84±0.01, 1.67±0.01 and 1.59±0.01 µg/dl respectively compared with control  
174 pH 6.0 values of 4.56±0.01 (leaf), 4.51±0.01 (stem) and 4.02±0.01 µg/dl (root). Results analysis also  
175 showed significant ( $P = .05$ ) reduction in vitamin C caused by SNAR and SSAR. Mean reduction values of  
176 9.48±0.01 (pH 2.0), 9.54±0.01 (pH 3.0), 10.05±0.01 (pH 4.0) for SNAR in leaf sample. While mean values  
177 of 9.68±0.01 (pH 2.0), 10.45±0.01 (pH 3.0), 11.40±0.01 (pH 4.0) were obtained for SSAR compared with  
178 control value of 23.80±0.01 mg/100 g (pH 6.0). Vitamin C values obtained for stem and root samples at

179 pH 2.0 and pH 4.0 were 11.78±0.01, 14.78±0.01 mg/100 g SNAR while values of 10.07±0.01, 10.21±0.01  
 180 and 13.78±0.01, 10.40±0.01 mg/100 g for SSAR plant organs as against control pH 6.0 values of  
 181 20.89±0.01 and 25.80±0.01 mg/100 g (Table 3).

182

183 **Table 3: Effect of simulated nitric and sulphuric acid on vitamin composition of plant parts of**  
 184 ***Amaranthus hybridus* L.**

|                                              | HNO <sub>3</sub> (pH Concs.) |           |            | H <sub>2</sub> SO <sub>4</sub> (pH Concs.) |            |            |               |
|----------------------------------------------|------------------------------|-----------|------------|--------------------------------------------|------------|------------|---------------|
| Vitamins                                     | 2.0                          | 3.0       | 4.0        | 2.0                                        | 3.0        | 4.0        | Control (6.0) |
| Vitamin A (β-Carotene)(µg/dl)                |                              |           |            |                                            |            |            |               |
| Leaf                                         | 2.20±0.01                    | 2.46±0.01 | 3.00±0.01  | 1.84±0.01                                  | 2.10±0.01  | 2.18±0.01  | 4.56±0.01     |
| Stem                                         | 2.18±0.01                    | 2.23±0.01 | 3.00±0.01  | 1.67±0.01                                  | 1.71±0.01  | 1.91±0.01  | 4.51±0.01     |
| Root                                         | 1.85±0.01                    | 1.92±0.01 | 2.01±0.01  | 1.59±0.01                                  | 1.59±0.01  | 1.59±0.01  | 4.02±0.01     |
| Vitamin B <sub>1</sub> (Thiamine) (mg/dl)    |                              |           |            |                                            |            |            |               |
| Leaf                                         | 1.10±0.01                    | 1.19±0.01 | 1.20±0.01  | 1.00±0.01                                  | 1.06±0.01  | 1.06±0.01  | 3.30±0.01     |
| Stem                                         | 1.08±0.01                    | 1.08±0.01 | 1.08±0.01  | 1.03±0.01                                  | 1.04±0.01  | 1.06±0.01  | 3.29±0.01     |
| Root                                         | 1.00±0.01                    | 1.02±0.01 | 1.02±0.01  | 1.00±0.01                                  | 1.01±0.01  | 1.02±0.01  | 3.15±0.01     |
| Vitamin B <sub>2</sub> (Riboflavin) mg/100 g |                              |           |            |                                            |            |            |               |
| Leaf                                         | 1.04±0.01                    | 1.08±0.01 | 1.09±0.01  | 1.18±0.01                                  | 1.22±0.01  | 1.26±0.01  | 1.79±0.01     |
| Stem                                         | 1.03±0.01                    | 1.04±0.01 | 1.04±0.01  | 1.17±0.01                                  | 1.20±0.01  | 1.20±0.01  | 1.78±0.01     |
| Root                                         | 1.03±0.01                    | 1.03±0.01 | 1.04±0.01  | 1.17±0.01                                  | 1.19±0.01  | 1.19±0.01  | 1.78±0.01     |
| Vitamin B <sub>3</sub> (Niacin)              |                              |           |            |                                            |            |            |               |
| Leaf                                         | 1.07±0.01                    | 1.09±0.01 | 1.10±0.01  | 1.08±0.01                                  | 1.12±0.01  | 1.29±0.01  | 1.60±0.01     |
| Stem                                         | 1.07±0.01                    | 1.08±0.01 | 1.10±0.01  | 1.06±0.01                                  | 1.10±0.01  | 1.20±0.01  | 1.58±0.01     |
| Root                                         | 1.07±0.01                    | 1.07±0.01 | 1.09±0.01  | 1.07±0.01                                  | 1.09±0.01  | 1.20±0.01  | 1.57±0.01     |
| Vitamin B <sub>6</sub> (Pyridoxin)           |                              |           |            |                                            |            |            |               |
| Leaf                                         | 1.30±0.01                    | 1.45±0.01 | 1.60±0.01  | 1.56±0.01                                  | 1.74±0.01  | 1.80±0.01  | 2.35±0.01     |
| Stem                                         | 1.24±0.01                    | 1.31±0.01 | 1.51±0.01  | 1.51±0.01                                  | 1.69±0.01  | 1.73±0.01  | 2.30±0.01     |
| Root                                         | 1.20±0.01                    | 1.25±0.01 | 1.40±0.01  | 1.46±0.01                                  | 1.55±0.01  | 1.55±0.01  | 2.26±0.01     |
| Vitamin E(α-Tocopherol)                      |                              |           |            |                                            |            |            |               |
| Leaf                                         | 0.39±0.01                    | 0.47±0.01 | 0.50±0.01  | 0.33±0.01                                  | 0.43±0.01  | 0.48±0.01  | 0.42±0.01     |
| Stem                                         | 0.39±0.01                    | 0.42±0.01 | 0.45±0.01  | 0.34±0.01                                  | 0.39±0.01  | 0.45±0.01  | 0.41±0.01     |
| Root                                         | 0.39±0.01                    | 0.42±0.01 | 0.46±0.01  | 0.34±0.01                                  | 0.38±0.01  | 0.44±0.01  | 0.42±0.01     |
| Vitamin C (Ascorbic acid)                    |                              |           |            |                                            |            |            |               |
| Leaf                                         | 9.48±0.01                    | 9.54±0.01 | 10.05±0.01 | 9.68±0.01                                  | 10.45±0.01 | 11.40±0.01 | 23.80±0.01    |

|      |            |            |            |            |            |            |            |
|------|------------|------------|------------|------------|------------|------------|------------|
| Stem | 11.78±0.01 | 13.13±0.01 | 14.78±0.01 | 10.07±0.01 | 11.28±0.01 | 13.78±0.01 | 20.89±0.01 |
| Root | 10.07±0.01 | 10.21±0.01 | 10.92±0.01 | 10.21±0.01 | 10.30±0.01 | 10.40±0.01 | 25.80±0.01 |

185 • Means of three replicates ± SEM,  $P = .05$ .

186 •  $\text{HNO}_3$  = Nitric acid,  $\text{H}_2\text{SO}_4$  = Sulphuric acid, Concs. = Concentrations

187 • SNAR = Simulated nitric acid rain, SSAR = Simulated sulphuric acid rain

188 **4.4 Effect of simulated nitric acid rain and sulphuric acid on antinutrients composition of plant**  
189 **parts of *Amaranthus hybridus* L.**

190 Statistical analysis of antinutrients results of *A. hybridus* parts depicted significant ( $P = .05$ ) decreases in  
191 phytate, soluble and total oxalate with increase in hydrocyanic acid of SNAR and SSAR parts over the  
192 control. Highest decreases were obtained at pH 2.0 of SNAR and SSAR and highest quantities of  
193 antinutrients at pH 6.0. Mean increase values for SNAR at pH 2.0 were 2.02±0.01, 2.03±0.01 and  
194 1.67±0.01 mg/100 g and for SSAR values of 2.30±0.01, 2.30±0.01 and 1.54±0.01 mg/100 g for leaf, stem  
195 and root respectively compared with control values of 1.60±0.01 (leaf), 1.60±0.01 (stem) and 1.10±0.01  
196 (root) (Table 4). Total oxalate had mean reduction values due to SNAR at pH 2.0 in leaf, stem and root  
197 parts of 22.04±0.01, 22.47±0.01 and 22.22±0.01 mg/100 g, SSAR had values of 24.91±0.01, 25.05±0.01  
198 and 33.22±0.01 mg/100 g respectively when compared with control pH 6.0 values of 38.86±0.01 (leaf),  
199 40.36±0.01 (stem) and 40.35±0.01 (root) (Table 4).

200

201

202

203

204 **Table 4: Effect of simulated nitric acid rain and sulphuric acid rain on antinutrient composition of**  
 205 **plant parts of *Amaranthus hybridus* L.**

| Antinutrients                 | mg/100 g                     |            |            |                                            |            |            |               |
|-------------------------------|------------------------------|------------|------------|--------------------------------------------|------------|------------|---------------|
|                               | HNO <sub>3</sub> (pH Concs.) |            |            | H <sub>2</sub> SO <sub>4</sub> (pH Concs.) |            |            | Control (6.0) |
|                               | 2.0                          | 3.0        | 4.0        | 2.0                                        | 3.0        | 4.0        |               |
| <b>Hydrocyanic acid (HCN)</b> |                              |            |            |                                            |            |            |               |
| Leaf                          | 2.02±0.01                    | 2.58±0.01  | 2.76±0.01  | 2.30±0.01                                  | 2.60±0.01  | 2.87±0.01  | 1.60±0.01     |
| Stem                          | 2.03±0.01                    | 2.28±0.01  | 2.55±0.01  | 2.30±0.01                                  | 2.60±0.01  | 2.75±0.01  | 1.60±0.01     |
| Root                          | 1.67±0.01                    | 1.74±0.01  | 2.06±0.01  | 1.54±0.01                                  | 1.63±0.01  | 1.86±0.01  | 1.10±0.01     |
| <b>Soluble oxalate</b>        |                              |            |            |                                            |            |            |               |
| Leaf                          | 18.12±0.01                   | 20.31±0.01 | 20.78±0.01 | 18.25±0.01                                 | 19.16±0.01 | 22.01±0.01 | 32.01±0.01    |
| Stem                          | 18.69±0.01                   | 20.31±0.01 | 20.75±0.01 | 18.24±0.01                                 | 19.15±0.01 | 22.22±0.01 | 32.00±0.01    |
| Root                          | 18.30±0.01                   | 20.30±0.01 | 20.75±0.01 | 22.66±0.01                                 | 24.11±0.01 | 25.00±0.01 | 32.00±0.01    |
| <b>Total oxalate</b>          |                              |            |            |                                            |            |            |               |
| Leaf                          | 22.04±0.01                   | 25.61±0.01 | 29.85±0.01 | 24.91±0.01                                 | 30.27±0.01 | 35.48±0.01 | 38.86±0.01    |
| Stem                          | 22.47±0.01                   | 28.16±0.01 | 30.62±0.01 | 25.05±0.01                                 | 36.00±0.01 | 37.34±0.01 | 40.36±0.01    |
| Root                          | 22.22±0.01                   | 29.32±0.01 | 34.34±0.01 | 33.22±0.01                                 | 35.12±0.01 | 37.16±0.01 | 40.35±0.01    |
| <b>Phytate</b>                |                              |            |            |                                            |            |            |               |
| Leaf                          | 74.25±0.01                   | 79.49±0.01 | 82.63±0.01 | 75.04±0.01                                 | 75.88±0.01 | 85.51±0.01 | 120.61±0.01   |
| Stem                          | 74.20 ±0.01                  | 79.38±0.01 | 81.53±0.01 | 75.04±0.01                                 | 75.87±0.01 | 84.63±0.01 | 120.30±0.01   |
| Root                          | 72.97±0.01                   | 76.38±0.01 | 80.44±0.01 | 73.36±0.01                                 | 73.38±0.01 | 74.76±0.01 | 120.25±0.01   |

206 • Means of three replicates ± SEM, *P* = .05.

207 • HNO<sub>3</sub> = Nitric acid, H<sub>2</sub>SO<sub>4</sub> = Sulphuric acid, Concs. = Concentration

208 • SNAR = Simulated nitric acid rain, SSAR = Simulated sulphuric acid rain

209

#### 210 4. Discussion

211 The effect of simulated nitric acid rain and sulphuric acid rain pH on the nutrient content of *Amaranthus*  
212 *hybridus* plant parts was determined. The findings of this research revealed a decrease in all the nutrients  
213 investigated. These nutrients perform essential body functions. A detailed report on the importance of  
214 these mineral nutrients (protein, minerals, vitamins, amino acids and antinutrients) to man has been given  
215 [16], [8].

216 Proximate parameters showed meaningful reductions to SNAR and SSAR treatments. Ash, protein, fat,  
217 fibre, carbohydrate and moisture contents of *A. hybridus* leaf, stem and root were significantly reduced  
218 due to SNAR and SSAR when compared with control (Table 1). These conform to reports by [17] who  
219 documented reduction in seed protein at lower pH levels for Amosy 71 (a cultivar) compared with control.  
220 The results of [18] showed that with increasing acidity of simulated acid rain, CAT (catalase) activity and  
221 P contents and soluble protein in the leaves of egg plant increased at first, reaching the highest at pH 4.0  
222 and 3.0, and then decreased; while sugar content and SOD (super oxide desmutase) activity and soluble  
223 sugar were adversely affected. [10] reported a reduction in the concentration of N in apple leaves at low  
224 pH. Nitrogen is a component of protein. The reduction in protein, fibre, fat and carbohydrate contents of  
225 *A. hybridus* by acid rain is threatening because of their role in health and wellbeing. Studies have  
226 indicated the preference of plant protein to animal protein since consumption of excessive animal protein  
227 is linked to heart disease and cancer. The suggestion is that meat should no longer be the centerpiece of  
228 a meal, but instead leafy vegetables (vegetarian) [19]. Plant fibre helps to maintain health and wellness.  
229 They reduce constipation and promote colon health, lower cholesterol, blood pressure and the risk of  
230 cardiovascular disease; and improve blood sugar control after a meal and aid in weight loss. They  
231 improve the absorption of minerals especially Ca. Fibre also helps to reduce diarrhea in some instances  
232 [20]. Ash content is very important from the biochemical nutritional view point. Ash contain all the  
233 important nutritional elements especially minerals (macro and microelements) which are very important  
234 for normal physiological body functions. Fat molecule consists fatty acids; they play an important role in  
235 insulin up-regulation and glucagon down-regulation. Fat is important from the nutritional view point  
236 because 1 g of fat give 9 Kcal of energy. Carbohydrate is the primary source of energy in the body; 1 g of  
237 carbohydrates yields 4 Kcal of energy. They are the principal and indispensable energy source. The RDA  
238 (Recommended Dietary Allowance) for carbohydrates is 130 g [21].

The mineral nutrients revealed decrease of nutrients in leaf, stem and root of *A. hybridus* occasioned by acid rain. All mineral nutrients; K, Na, Ca, Mg, Fe, Zn, Cu, Mn were significantly ( $P = .05$ ) reduced in acid treated plant parts compared with the control. The reason for this decrease may be due to the negatively charged sulphate and nitrate ions in the acid acting as inhibitors permitting positive ions to be leached out of the soil. As nutrients are leached out of the soil owing to simulated acid rain the plants do not have enough nutrients for growth and to build up their own nutrients resulting in a decrease in nutrients content. Magnesium and iron are important components of chlorophyll, their reduction causes chlorosis and leaf chlorosis compromised the photosynthetic capacity of the plant thus, affecting the plant performance. The reduction in these minerals occasioned by acid rain is worrisome because when minerals are deficient in diet, the body picks up toxic metals from the environment. Minerals are important and cannot be manufactured within the body but must be obtained daily in plants diet. Because of our exposure to toxic metals and toxic chemicals, it is advisable to eat plenty of these vital minerals from plants (*A. hybridus*) in order to reduce the accumulation of toxic metals from our bodies [16].

This research also revealed significant reductions in vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, E and C content of *A. hybridus* induced by SNAR and SSAR. Reductions due to acid rain in some strawberry vitamins have been reported [9]. There are various reports on changes in vitamin levels due to various stress factors [22], [23]. [24] reported a decrease in pear fruit vitamin C caused by simulated acid rain under pH 4.0. The formation of free radical oxygen is indispensable in living organisms using oxygen gases in their metabolism. It is well established that factors in the environment that cause stress increase free radical formation, to remove this free radical effect organisms must develop various defense mechanisms. [25] documented that acid rain cause a decrease in protein content and an increase in oxygen radicals in their organs. Vitamins reductions in this study may be due to increase in the free radical formation in the plant due to acid rain stress, and plant's use of the vitamins in order to resist the stress which results in a loss in vitamin levels. Reductions may also be due to the fact that in autotrophy crops metabolic ways formed by vitamin synthesis are somewhat inhibited by acidic conditions. It is an established fact that various crops exposed to stress change their metabolism.

## 5. Conclusion

Data obtained in this study revealed that SNAR and SSAR treatments caused adverse reductions in the nutrient content of *Amaranthus hybridus* L. Reductions were significantly different from one pH concentration to the other in both simulated nitric acid rain ( $\text{HNO}_3$ ) and simulated sulphuric acid rain ( $\text{H}_2\text{SO}_4$ ). Nutrients reduction was high at pH 2.0 and low at pH 4.0 compared with the control pH 6.0. Simulated  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  acid rain caused significant reductions in nutrient content of *A. hybridus*. Control plant samples had the highest amount of all the nutritional parameter investigated. The levels of nutrients in parts of *A. hybridus* differ. The reductions induced by SNSAR on the nutrients of *A. hybridus* call for urgent attention because plant nutrients are effective in the prevention and treatment of various health conditions such as cancer, heart diseases, diabetes, high blood pressure etc, and they remain the only way of obtaining natural and cheapest sources of mineral nutrients [26]. There is therefore, the need to prevent *A. hybridus* from acid rain effects.

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