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Physiological and Morphological Responses of *Amaranthus hybridus* L. (Green) to Simulated Nitric and Sulphuric Acid Rain

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Authors' contributions

This work was carried out in collaboration between both authors. Author AAJM designed the study, performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author LS handled the data collection in the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the physiological and morphological responses of *Amaranthus hybridus* leaf area, shoot height, stem girth, leaf number, petiole length, fresh weight (FW) and dry weight (DW) of leaf, shoot and root, relative growth rate (RGR) and chlorophyll (chl.) content to simulated nitric and sulphuric acid rain.
Place and Duration of Study: Department of Botany, University of Calabar, Calabar, Nigeria, between February and April, 2016.
Methodology: Thirty five poly bags were used. Simulated nitric and sulphuric acid rain (SNAR and SSAR) of pH 2.0, 3.0, 4.0 and a control pH of 6.0 were separately prepared and sprayed every two days. The research was carried out in a greenhouse under controlled conditions.
Results: Results showed highest decreases at pH 2.0 and lowest decreases at pH 4.0 in all the physiological parameters studied. Highest decreases are depicted by lowest measured values while lowest decreases by highest values in all measured parameters as affected by SNAR and

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SSAR. Acid rain treated plants showed necrosis, chlorosis and leaf deformation. Chlorophyll a, b and total chlorophyll of acid rains treated plants revealed a trend of decrease in content with increasing period of development. Mean values for leaf area response to simulated HNO_3 and H_2SO_4 acid rains of pH 2.0, pH 3.0 and pH 4.0 at 4 weeks period of development were 14.60±0.33^d, 17.50±0.47^d, 18.80±0.11^d and 14.94±0.23^d 17.70±0.20^d, 17.92±0.28^d as against control value of 22.62±0.26^d cm^2. Mean values for shoot height response to acid rains had values of 16.48±0.59^d, 19.65±0.66^d, 20.46±0.88^d, 15.82±0.59^d and 18.27±0.12^d, 19.74±0.17^d and 24.48±0.23^d cm. Mean values for chl. a, b and total chl. at 28 days for SNAR and SSAR pH 2.0 and pH 6.0 were 18.9±0.12, 23.4±0.04 mg g^{-1} FW and 42.3, 20.0±0.3, 23.6±0.3 mg g^{-1} FW and 43.8 and 58.2 0.3, 71.2 0.3 mg g^{-1} FW and 129.4 respectively.

Conclusion: Physiological and morphological parameters studied responded negatively to simulated nitric acid (HNO_3) and sulphuric acid (H_2SO_4) rain with significant decrease at all acidity levels with respect to the control.

Keywords: Amaranthus hybridus; physiological responses; simulated nitric and sulphuric acid rain; growth; chlorophyll.

1. INTRODUCTION

The quality of air is of great importance for all living things. The health of plant, animal and human depends on a clean atmosphere. Human activities have released into the air elements that have the ability to cause pollution such as sulphur dioxide (SO_2), oxides of nitrogen (NO_x), carbon dioxide (CO_2) and hydrogen fluoride (HF) producing acid deposition (acid rain) as a result of complex physical and chemical reactions. Most of these reactions are accelerated by sunlight. The transportation of compounds, which convey acid rains through the prevailing wind for thousands of miles raises the pollution to very high rates. Sulphuric acid and nitric acid are the most important components of acid rain derived largely from fossil fuels combustion [1]. Acid rain occurs in nature. In Europe some dramatic effects of acid rain on forest have been observed. A survey in West Germany showed that in 1983, 34% of the country's total forest including half of the famous Black Forest was damaged by air pollution. Damage of 14% forest trees was recorded in Switzerland. Startling evidence of tree damage was observed on Carmels Hump in Vermont's Green Mountains with Conifers being the most affected because the needles are bathed in acid droplets all year around. Other trees drop their leaves. Balsam fir and red spruce had a decline in biomass of 20% and 73% from 1965 to 1983. Sugar maples and beech trees lower on the mountain revealed biomass drop of 25% [Charles 2003]. Acid rain is a threat to forests. In Nigeria, forests in the oil producing areas are badly affected by acid rain. Acid rain exhibited significant destruction of the forest. Plants which are the primary producer are affected much by acid rain [2]. Pollutants get deposited on the surface of plants and interfere with photosynthesis plant vital process. This abruptly causes death of plants. Acid deposition due to rainfall has the potential to affect sensitive forest. Acid rain directly impact forest ecosystem and their inhabitants. It also decreases the growth of forest trees (Osu and Expo 2013). A number of studies have shown that acid rains have serious effects on vegetative organ and generative structure [3,4]. Acid rain induces changes in cellular biochemistry and whole plant physiology. Biological effects of acid rain deposition on plants are numerous and complex, and include symptoms of visible injury such as necrosis and chlorosis as well as invisible effects of reduced photosynthesis, nutrient loss from leaves, alteration in water balance, variation of several enzyme activities [5]. Exposure of plants to acid rain caused structural changes in photosynthetic pigment apparatus and a decrease in chlorophyll concentrations.

Acid rain can also be simulated in laboratories and greenhouses. The negative effects of simulated acid rain on morphological and physiological parameters (plant growth characteristics) have been reported. A decreased in shoot length, number of leaves, petiole length and leaf area of *Amaranthus hybridus* and *Abelmoschus esculentus* plants treated with simulated acid rain [6]. Simulated acid rain caused a decreased in plant height, shoot length, root length, number of lateral branches, number of leaves, number of floral bud, number of flowers, number of bud and seed yield in *Solanum melongena* treated with simulated acid rain [7]. Under the stress of simulated acid rain, growth parameters such as leaf number, shoot height, fresh and dry weight and stem girth were significantly reduced. Simulated acid rain also induced morphological changes such as chlorosis and necrosis in *Solanum lycopersicum* [8]. Effect of simulated acid rain on *Shorea macroptera* resulted in seedling height reduction, a decrease in dry matter production, chlorosis and necrosis [9]. Simulated acid rain induced morphological changes including chlorosis, early leaf senescence, necrosis, leaf abscission, leaf folding and death. Acid rain decreased plant height, leaf area, fresh weight, relative growth rate, chlorophyll content of leaf and harvest index of *Vigna unguiculata* [10]. Reduction in number of leaves, shoot: root, shoot water content and potassium ion concentration in *Vigna mung* occurred due to acid rain [11]. *Acacia nilotica* showed deleterious morphological and growth characters when exposed to simulated acid rain [12].

Amaranthus hybridus L. is an important vegetable crop in Nigeria. Widely cultivated and consumed in the southern parts of the country. It is a crop of commercial importance as it generates income to the growers. The vegetable constitutes a major part of the diet of people in the middle and southern Nigeria where they are used mostly in the preparation of soup. Nutritional composition of this vegetable reveals high amount of protein, fat, fibre, ash, mineral elements, vitamins and amino acids. The diet of the poor people in Nigeria and other developing countries of the world are dominated by highly starchy foods thus, vegetables are indispensable constituent in the diet of the people.

Acid rain has been reported to have deleterious effect on the growth of plants. The present study seeks to assess some physiological and morphological responses of *Amaranthus hybridus* to simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR).

2. MATERIALS AND METHODS

2.1 Seed Collection

Seeds of *Amaranthus hybridus* were obtained from farmers in the University Staff Quarters and grown in the Department of Botany Greenhouse located in the Botanical Garden of the University of Calabar, Calabar, Nigeria (latitude 4.952'N and longitude 8.341'E) at 25±3℃. On germination, the young seedlings were transferred into poly bags and allowed to stay for a week with regular water application. Planting was done in the month of Feburary 2016. Actual growth measurement started in March and ended in April (eight weeks).

2.2 Simulated Acid Rain Preparation

Simulated nitric and sulphuric acid rain (SNAR and SSAR) were separately prepared. Each pH concentration (HNO_3 or H_2SO_4) was prepared using different amount of acid. Different pH concentrations of 2.0, 3.0 and 4.0 were used in this study. Thirty (30 ml) of each acid was used for pH concentration of 2.0, 20.1 ml for pH 3.0 and 10.2 ml for pH 4.0 using deionized water with a digital pH meter (WTW 330). Deionized water of pH 6.0 was used as control (Mofunanya's method). Thirty five (35) poly bags; (fifteen bags for simulated nitric acid rain, fifteen bags for sulphuric acid rain and five bags for the control) that is five for each pH concentration replicated three times. Prior to acids rain application, the bags were arranged in a randomized block design. Simulated acid rain of different concentrations was applied three times in a week using a medium size pressurized sprayer on the plants.

2.3 Growth Measurement

Various parameters were used in assessing the growth of *A. hybridus* treated with SNAR and SSAR; leaf area, shoot length, stem girth, number of leaves produced, petiole length, relative growth rate (RGR), fresh weight of leaves, shoots and roots. Leaf area was determined by placing SNAR and SSAR leaf each on a 1 mm^2 graph paper as well as control leaf. The size of the leaf was traced on the paper and the total area calculated on the basis of the number of squares covered within the region. Shoot height was measured using a tape rule in (cm) from the soil level to the terminal bud. Shoot height measurements were taken at a weekly interval for eight weeks post-treatments. Stem girth was measured with the aid of the vernier caliper. Leaves were counted weekly to ascertain the number of leaves produced. Petiole length was obtained by measuring from leaf bud to the leaf base. At the termination of experiment, the leaves, shoots and roots were harvested and weighed (FW) before drying to constant weight (DW) (Biomass). The RGR was calculated following the method of [13] and the fresh weight of whole plant was used in the determination of RGR.

$RGR = (\log W_1 - W_2)/ (T_2-T_1)$

Where;

W_2 = final weight
W_1 = initial weight
T_2 = final time
T_1 = initial weight

2.4 Chlorophyll Content Determination

The chlorophyll content of leaves was determined by the method of [14] using the formula

Chl a = (11.6 A663-1.3 A643) VX^{-1}
Chl b = (19.6 A643-4.7 A663) VX^{-1}
Chl a + b = (mg g^{-1} FW)

Where, A663 and A643 are absorbance at 663 and 643 nm, respectively. A = absorbance, V^{-1}= volume of 80% acetone, X^{-1} = sample fresh weight, mg = milligram and FW = fresh weight.

Chlorophyll measurements were taken at 14 days post-treatment for two months.

2.5 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]. Treatment means are separated using the Duncan Multiple Range Test [16].

3. RESULTS

3.1 Response of *Amaranthus hybridus* Leaf Area to Simulated Nitric Rain (SNAR) and Sulphuric Acid Rain (SSAR)

There were significant (P = .05) reductions in leaf area response to simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) of pH 2.0, 3.0 and 4.0 throughout the period of development with lowest reductions obtained at pH 2.0. Significant (P = .05) reductions in leaf area response to SNAR at weeks 5 had mean values of 18.96 ± 1.75^d (pH 2.0), 21.08 ± 0.61^e (pH 3.0), 21.48 ± 0.66^e (pH 4.0) and values of 18.62 ± 0.34^d (pH 2.0), 21.10 ± 0.36^e (pH 3.0), 21.26 ± 0.54^e (pH 4.0) for SSAR compared to control value of 27.88 ± 0.29^e. Leaf area of *A. hybridus* was significantly (P = .05) affected by SNAR and SSAR (Table 1). Lowest leaf area value at all developmental periods indicates highest reductions.

3.2 Shoot Height Response of *Amaranthus hybridus* to SNAR and SSAR

Results presented in (Table 2) revealed significant (P = .05) reduction in shoot height in simulated nitric and sulphuric acid rain treated plants with lowest reduction occurring at pH 2.0. Shoot height response at week one for plants treated with simulated HNO_3 and H_2SO_4 acid rain did not differ from the control. Shoot height response of acids treated and control plants showed increase with increasing period of development with acids rain treated plants having more reduction in shoot height when compared to the control. Simulated sulphuric acid caused more reductions in shoot height than SNAR. Shoot heights of acid rain treated plants varied according to acidity levels or concentrations with pH 2.0 causing more reductions than pH 3.0, and pH 3.0 more than pH 4.0. Shoot height reductions caused by SNAR at 5 weeks developmental period were 20.80 ± 1.74^e (pH 2.0), 22.16 ± 1.6^e (pH 3.0) and 24.40 ± 1.46^e cm (pH 4.0) while SSAR had values of 20.00 ± 0.54^e (pH 2.0), 21.68 ± 0.52^e (pH 3.0) and 22.11 ± 0.60^e cm (pH 4.0) as against control (pH 6.0) value of 41.01 ± 0.89^e cm.

3.3 Response of *Amaranthus hybridus* Stem Girth to Simulated Nitric and Sulphuric Acid Rain

Acids rain treated plants had the lowest stem girth while control plants had the highest stem girth. Significant (P = .05) reductions in stem girth at week 1 and 8 were 0.25 ± 0.04^a, 0.44 ± 0.03^c mm (pH 2.0) for SNAR and 0.25 ± 0.03^a, 0.43 ± 0.03^c mm (pH 2.0) for SSAR compared to control (pH 6.0) values of 0.37 ± 0.23^a, 1.17 ± 0.03^f mm. Results have shown significant reductions in stem girth of *A. hybridus* according to concentrations (Table 3).

3.4 Response of *Amaranthus hybridus* Leaf Number to Simulated Nitric and Sulphuric Acid Rain

Results of *A. hybridus* leaf number response to acids rain revealed a significant (P = .05) decrease in number of leaves produced. The plants had the highest leaf number production at the control (pH 6.0) compared to SNAR and SSAR of pH 2.0, pH 3.0 and pH 4.0. At week 1, leaf number production in acids treated was similar to the control plants. Significant (P = .05) decreases in number of leaf produced for SNAR and SSAR pH 2.0, 3.0 and 4.0 at week 2 had values of 4.80±0.37^{a}, 4.60±0.24^{a}, 4.40±0.24^{a} and 4.40±0.24^{a}, 4.60±0.24^{a}, 4.80±0.24^{a} compared with control value of 6.80±0.37^{b} (pH 6.0). At week 8 values were 20.00±0.54^{f}, 22.20±0.37^{f}, 23.20±0.24^{f} and 19.60±0.40^{g}, 20.20±0.37^{g}, 20.40±0.20^{g} and control value of 29.00±0.31^{h} (pH 6.0) respectively. Reduction in leaf number production of *Amaranthus hybridus* revealed a negative response to SSAR and SNAR (Table 4).

Table 1. Response of *Amaranthus hybridus* leaf area to simulated nitric rain (SNAR) and sulphuric acid rain (SSAR)

T (Wks)	Cm^2 HNO_3 (pH Concs.)			Cm^2 H_2SO_4 (pH Concs.)			Cm^2 Control
	2.0	**3.0**	**4.0**	**2.0**	**3.0**	**4.0**	**6.0**
1	4.26±0.11^{a}	4.28±0.19^{a}	4.30±0.14^{a}	4.22±0.09^{a}	4.24±0.10^{a}	4.26±0.10^{a}	5.36±0.18^{a}
2	6.12±0.16^{b}	6.88±0.12^{b}	7.08±0.32^{b}	5.96±0.35^{a}	6.94±0.40^{b}	6.98±0.91^{b}	8.64±0.18^{b}
3	12.58±0.11^{c}	13.70±0.32^{c}	14.12±0.15^{c}	12.80±0.39^{b}	13.16±0.26^{c}	13.22±0.36^{c}	15.70±0.40^{c}
4	14.60±0.33^{d}	17.50±0.47^{d}	18.80±0.11^{d}	14.94±0.23^{c}	17.70±0.20^{d}	17.92±0.28^{d}	22.62±0.26^{d}
5	18.96±1.75^{d}	21.08±0.61^{e}	21.48±0.66^{e}	18.62±0.34^{d}	21.10±0.36^{e}	21.26±0.54^{e}	27.88±0.29^{e}
6	29.22±1.24^{e}	30.00±0.95^{f}	33.54±0.76^{f}	29.12±0.70^{e}	31.00±0.57^{f}	31.28±0.48^{f}	38.52±0.64^{f}
7	32.22±1.26^{f}	33.00±0.95^{f}	35.50±0.73^{f}	31.12±0.70^{f}	33.00±0.57^{f}	34.48±0.25^{f}	47.52±0.64^{g}
8	35.22±1.26^{f}	36.00±0.95^{f}	37.50±0.73^{f}	35.00±0.21^{f}	35.00±0.21^{f}	36.48±0.25^{f}	56.52±0.64^{h}

- *Values are mean ± standard error, n = 5, P = .05.*
- *T (Wks) =Treatment weeks*
- *Means followed by same superscript letters in each column are not significantly different, while means followed by different superscript letters are significantly different at (P = .05) by Duncan Multiple Range Test*

Table 2. Response of *Amaranthus hybridus* shoot height to simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR)

T (Wks)	Cm HNO_3 (pH Concs.)			Cm H_2SO_4 (pH Concs.)			Cm Control
	2.0	**3.0**	**4.0**	**2.0**	**3.0**	**4.0**	**(6.0)**
1	5.32±0.66^{a}	5.36±0.64^{a}	5.39±0.59^{a}	5.28. ±0.26^{a}	5.29±0.11^{a}	5.33±0.14^{a}	5.66±0.26^{a}
2	10.50±0.50^{b}	11.06±0.39^{b}	11.26±0.31^{b}	10.47±0.20^{b}	10.73±0.27^{b}	10.91±0.41^{b}	13.20±0.21^{b}
3	13.98±0.25^{c}	14.13±0.56^{c}	15.31±0.51^{c}	12.51±0.30^{c}	13.78±0.34^{c}	14.02±0.71^{c}	18.30±0.22^{c}
4	16.48±0.59^{d}	19.65±0.66^{d}	20.46±0.88^{d}	15.82±0.59^{d}	18.27±0.12^{d}	19.74±0.17^{d}	24.48±0.23^{d}
5	20.80±1.74^{e}	22.16±1.22^{e}	24.40±1.46^{e}	20.00±0.54^{e}	21.68±0.52^{e}	22.11±0.60^{e}	41.01±0.89^{e}
6	29.12±1.30^{f}	30.88±1.40^{e}	31.43±1.21^{f}	31.43±1.21^{f}	28.68±1.03^{f}	29.93±1.11^{f}	49.58±0.80^{f}
7	34.35±1.11^{g}	35.01±1.01^{g}	36.00±1.00^{g}	31.02±1.21^{g}	31.75±1.16^{g}	32.99±1.04^{g}	52.60±0.80^{g}
8	37.32±1.46^{h}	38.51±1.40^{h}	40.22±1.53^{h}	33.05±1.16^{h}	33.79±1.01^{h}	34.10±1.12^{h}	55.74±0.85^{h}

- *Legend is similar to that in Table 1.*

Table 3. Response of *Amaranthus hybridus* stem girth to simulated nitric and sulphuric acid rain (SNAR and SSAR)

T (Wks)	Mm HNO_3 (pH Concs.)			Mm H_2SO_4 (pH Concs.)			Mm Control
	2.0	**3.0**	**4.0**	**2.0**	**3.0**	**4.0**	**(6.0)**
1	0.25±0.04^{a}	0.28±0.03^{a}	0.33±0.03^{a}	0.25±0.03^{a}	0. 28±0.03^{a}	0.33±0.03^{a}	0.37±0.23^{a}
2	0.27±0.41^{a}	0.30±0.03^{a}	0.35±0.03^{a}	0.26±0.04^{a}	0.29±0.11^{a}	0.34±0.03^{a}	0.70±0.03^{b}
3	0.38±0.03^{b}	0.43±0.03^{b}	0.45±0.03^{b}	0.35±0.06^{b}	0.42±0.03^{b}	0.44±0.47^{b}	0.81±0.03^{c}
4	0.40±0.43^{c}	0.46±0.03^{b}	0.47±0.01^{b}	0.41±0.03^{c}	0.43±0.03b^{c}	0.46±0.03^{bc}	0.90±0.03^{d}
5	0.42±0.13^{c}	0.47±0.23^{b}	0.47±0.03^{b}	0.41±0.03^{c}	0.46±0.03b^{c}	0.46±0.03^{bc}	0.96±0.03^{e}
6	0.43±0.03^{c}	0.50±0.03^{c}	0.51±0.03^{c}	0.42±0.03^{c}	0.47±0.03b^{c}	0.49±0.03^{bc}	1.07±0.03^{f}
7	0.43±0.03^{c}	0.50±0.03^{c}	0.51±0.03^{c}	0.42±0.03^{c}	0.48±0.03b^{c}	0.49±0.03^{bc}	1.11±0.03^{f}
8	0.44±0.03^{c}	0.53±0.03^{c}	0.53±0.03^{c}	0.43±0.03^{c}	0.49±0.03^{c}	0.51±0.03^{c}	1.17±0.03^{f}

- *Legend is similar to that in Table 1*

Table 4. Response of *Amaranthus hybridus* leaf number to simulated nitric and sulphuric acid rain (SNAR and SSAR)

T	HNO_3 (pH Concs.)			H_2SO_4 (pH Concs.)			Control
(Wks)	2.0	3.0	4.0	2.0	3.0	4.0	(6.0)
1	4.00±031^{a}	4.00±0.31^{a}	4.00±0.20^{a}	4.00±0.20^{a}	4.00±0.20^{a}	4.00±0.20^{a}	4.00±0.00^{a}
2	4.80±0.37^{a}	4.60±0.24^{a}	4.40±0.24^{a}	4.40±0.24^{a}	4.60±0.24^{a}	4.80±0.24^{a}	6.80±0.37^{b}
3	10.00±0.70^{b}	10.00±0.70^{b}	10.60±0.67^{b}	9.60±0.67^{b}	10.10±0.67^{b}	10.10±0.50^{b}	13.40±0.24^{c}
4	13.80±0.58^{c}	14.60±0.50^{c}	14.80±0.37^{c}	12.40±0.24^{c}	12.60±0.24^{c}	12.90±0.24^{c}	15.60±0.24^{d}
5	14.60±0.50^{d}	16.60±0.24^{d}	16.80±0.24^{d}	13.80±0.48^{d}	14.40±0.58^{d}	14.80±0.24^{d}	20.40±0.50^{e}
6	17.00±0.54^{e}	18.20±0.37^{e}	18.40±0.24^{e}	15.60±0.40^{e}	16.20±0.40^{e}	16.40±0.37^{e}	23.00±0.31^{f}
7	19.00±0.54^{f}	21.20±0.37^{f}	22.40±0.24^{f}	17.60±0.40^{f}	18.00±0.37^{f}	18.20±0.20^{f}	25.20±0.31^{g}
8	20.00±0.54^{f}	22.20±0.37^{f}	23.20±0.24^{f}	19.60±0.40^{g}	20.20±0.37^{g}	20.40±0.20^{g}	29.00±0.31^{h}

- *Legend is similar to that in Table 1.*

3.5 Response of *Amaranthus hybridus* Petiole Length to Simulated Nitric and Sulphuric Acid Rain

Amaranthus hybridus petiole length showed negative response to SNAR and SSAR with significant (*P* = .05) reductions at all stages of growth (Table 5). Results varied according to acids concentrations. Reductions were highest at (pH 2.0) and lowest at (pH 4.0) when compared to control (pH 6.0). Highest and lowest mean values reductions for SNAR and SSAR at week 1 and 8 were 6.61±0.47^{f}, 1.54±0.81^{a} (pH 2.0), 5.82±0.42^{e}, 1.53±0.11^{a} cm (pH 2.0) as against values of 1.89±0.03^{a}, 9.04±0.34^{g} cm for control pH 6.0. Petiole reductions were more with SSAR than with SNAR.

3.6 Responses of *Amaranthus hybridus* FW and DW (Leaf, Shoot and Root) to Simulated Nitric and Sulphuric Acid Rain

Plant biomass showed negative response to SNAR and SSAR. A drastic reduction in biomass was observed for acid-rain treated plants, compared to the control. Leaf fresh and dry weight of nitric acid (HNO_3) rain treated plants at pH 2.0 had reductions of 16.99±0.11^{e}, 2.03±0.03^{b} g while sulphuric acid (H_2SO_4) rain treated plants had values of 16.91±0.03^{d}, 2.01±0.06^{b} g. Shoot FW and DW at pH 2.0 had mean reduction values of 17.27±0.03^{e}, 16.99±012^{d} (SNAR) and 16.89±0.12^{b}, 2.94±0.03^{b} g (SSAR) compared to control (pH 6.0) values of 29.10±0.05^{f} and 7.34±0.01^{b} g. A similar trend was obtained for roots FW and DW which had reduction values at pH 2.0 and pH 6.0 of 5.05±0.11^{c}, 1.08±0.01^{a}, 5.00±0.02^{c}, 1.02±0.06^{a} and 6.01±0.13^{c}, 5.98±0.01^{d}g respectively. Highest plant biomass reductions occurred at pH 2.0 than pH 3.0 and pH 4.0 compared to the control (Table 6).

3.7 Responses of *Amaranthus hybridus* Relative Growth Rate (RGR) to Simulated Nitric Rain (SNAR) and Sulphuric Acid Rain (SSAR)

The relative growth rate of acid rain treated plants (pH 2.0, pH 3.0 and pH 4.0) was severely affected compared to the control pH 6.0. A trend of decrease in RGR with progressive period of growth was observed in both acid rain treated and control plants with more reductions obtained for SNAR and SSAR plants than the control plants. Significant (*P* = .05) mean reductions values for RGR at day 14 at pH 2.0, pH 3.0 and pH 4.0 were 50 x 10^{-4}±0.12^{d}, 60 x 10^{-4}±0.6^{d}, 69 x10^{-4}±0.21^{d} and 49 x10^{-4}±1.3^{d}, 54 x10^{-4}±0.18^{d} and value of 63 x10^{-4}±0.3^{d} $gg^{-1}day^{-1}$ pH 6.0 respectively. Corresponding values at day 56 were 26 x 10^{-4}±0.4^{a}, 35 x 10^{-4}±0.1^{a}, 39 x 10^{-4}±2.2^{a} and 25 x 10^{-4}±1.6^{a}, 34 x 10^{-4}±1.2^{a}, 38 x 10^{-4}±1.2^{a} and 50 x 10^{-4}±1.7^{a} $gg^{-1}day^{-1}$ (pH 6.0) (Table 7).

3.8 Responses of *Amaranthus hybridus* Chlorophyll (Chl.) Content to Simulated Nitric and Sulphuric Acid Rain

Statistical analysis of data regarding chl. a, b and total chl. contents depicted significant (*P* = .05) decreases at all stages of development in acid rain treated plants at pH 2.0, 3.0 and 4.0 compared with the control (pH 6.0). A general trend of decrease in chl. content in SNAR and SSAR treated plant with increasing period of development and increase in chl. content with increasing period of development in the control plants was obtained. Chlorophyll a, b and total chl. responded negatively to SNAR and SSAR with reductions in chl. content as shown in (Table 8). Results varied according to simulated acids concentrations. At 14 days period of development, significant (*P* = .05) mean reduction values of 24.1 ± 0.3^{d}, 28.4 ± 0.06^{d}, 52.5^{d} (pH 2.0), 29.5 ± 0.2^{d}, 34.6 ± 0.3^{d}, 64.1^{d} (pH 3.0,) 37.6 ± 0.06^{d}, 44.2 ± 0.3^{d}, 81.8^{d} (mg g^{-1} FW) (pH 4.0) were obtained for SNAR. Corresponding values for SSAR at pH 2.0 were 24.0 ± 0.03^{d}, 27.8 ± 0.3^{d}, 51.8^{d}, pH 3.0 were 27.6 ± 0.03^{d}, 32.5 ± 0.3^{d}, 60.1^{d} and values of 33.7 ± 0.3^{d}, 39.9 ± 0.3^{d}, 73.6^{d} (mg g^{-1} FW) respectively for SSAR compared to 45.4 ± 0.1^{a}, 58.3 ± 0.3^{a}, $1027.6\pm0.03^{d}3.7^{a}$ (mg g^{-1} FW) for control (pH 6.0) respectively at 14 days period of development. Chl. content reduction values at 56 days period at pH 2.0 when compared to control (pH 6.0) were 17.8 ± 0.1^{a}, 20.0 ± 0.03^{a}, 37.8^{a} and 17.0 ± 0.43^{a}, 19.7 ± 0.3^{a}, 36.7^{a}(mg g^{-1} FW) for SNAR and SSAR with values of 74.8 ± 0.01^{d}, 89.1 ± 0.01^{d}, 163.9^{d}(mg g^{-1} FW) for the control.

4. DISCUSSION

Acid rain cause great damage crop plants as well as vegetable.

Physiological and morphological characteristics of *Amaranthus hybridus* L. were found to be affected by simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR). *Amaranthus hybridus* responses varied according to acids concentrations with pH 2.0 having the lowest values followed by pH 3.0 and then pH 4.0. Lowest values depicted highest reductions, followed by lower and low values in all the growth parameters, chlorophyll contents and yield loses studied. Although the effects of different acids concentrations varied significantly, there was no tenfold change in values from pH 2.0 to pH 3.0 and from pH 3.0 to pH 4.0. Plants respond differently to stress factors in their growing environment. All forms of stress (drought, acids, pathogens, salinity etc.) triggers a series of physiological, morphological and anatomical adjustment in order for the plants to cope with various changes induced by stress. All the physiological parameters investigated; leaf area, shoot height, stem girth, number of leaves produced, petiole length, leaf, shoot and root FW and DW, relative growth rate and chlorophyll content of *A. hybridus* were significantly decreased at all acidity levels with respect to the control. Results of this study show that the higher the acidity of SNAR and SSAR treatment, the more adverse the effects were on the measured physiological parameters. Leaf area response of *A. hybridus* to SNAR and SSAR showed reductions at different pH concentrations. The reduction in leaf size or area was due to thinner mesophyll cells. Reduction in leaf area conforms to the observation of [8]. Shoot height showed negative response resulting in reductions due to SNAR and SSAR. The value showed a role of different acidity levels in the reduction of plant height. Stem girth was smaller in acids rain treated plants than in the control. Decrease in stem girth corresponded to increasing acidity. The result is similar to earlier result [17].

Amaranthus hybridus exposure to simulated nitric and sulphuric acid rain decreased the number of leaves produced by acid rain treated plants when compared to the control plants. This effect could come from stress mechanism of leaves exposed to different acid concentrations. According to the report of [18], leaf growth is affected by simulated acid rain due to reduction in transpiration and essential nutrient uptake. Results of this study indicated that under simulated acid rain stress, shoot height and number of leaves decreased with declining pH concentrations of acid rains, which affects the terminal buds of plants. This is similar to the work of [19].

Table 5. Response of *Amaranthus hybridus* petiole length to simulated nitric rain (SNAR) and sulphuric acid rain (SSAR)

T (Wks)	Cm HNO_3 (pH Concs.)			Cm H_2SO_4 (pH Concs.)			Cm Control
	2.0	**3.0**	**4.0**	**2.0**	**3.0**	**4.0**	**(6.0)**
1	1.54 ± 0.81^{a}	1.55 ± 0.15^{a}	1.55 ± 0.58^{a}	1.53 ± 0.11^{a}	1.54 ± 0.14^{a}	1.55 ± 0.14^{a}	1.89 ± 0.03^{a}
2	2.14 ± 0.15^{b}	2.36 ± 0.14^{b}	2.67 ± 0.10^{b}	2.10 ± 0.22^{b}	2.21 ± 0.11^{b}	2.34 ± 0.19^{b}	3.38 ± 0.17^{b}
3	3.34 ± 0.25^{c}	3.84 ± 0.22^{c}	4.02 ± 0.86^{c}	2.78 ± 0.20^{b}	3.01 ± 0.08^{c}	3.70 ± 0.13^{c}	5.32 ± 1.90^{c}
4	4.24 ± 0.17^{d}	4.50 ± 0.19^{d}	4.44 ± 0.16^{c}	3.65 ± 0.07^{c}	3.71 ± 0.10^{c}	3.90 ± 0.10^{c}	6.64 ± 0.22^{d}
5	4.56 ± 0.28^{d}	4.86 ± 0.32^{d}	5.01 ± 0.16^{d}	4.00 ± 0.07^{d}	4.11 ± 0.10^{d}	4.20 ± 0.16^{d}	7.64 ± 0.20^{e}
6	5.20 ± 0.32^{e}	5.55 ± 0.33^{e}	5.71 ± 0.26^{d}	4.54 ± 0.32^{d}	4.78 ± 0.10^{d}	4.84 ± 0.11^{d}	7.86 ± 0.26^{e}
7	6.01 ± 0.32^{f}	6.21 ± 0.10^{f}	6.95 ± 0.24^{e}	5.10 ± 0.32^{e}	5.58 ± 0.33^{e}	5.75 ± 0.42^{e}	8.46 ± 0.30^{f}
8	6.61 ± 0.47^{f}	6.75 ± 0.10^{f}	7.89 ± 0.29^{f}	5.82 ± 0.42^{e}	5.94 ± 0.24^{e}	5.99 ± 0.14^{e}	9.04 ± 0.34^{g}

- *Legend is similar to that in Table 1.*

Table 6. Responses of *Amaranthus hybridus* fresh weight (FW) and dry weight (DW) (leaf, shoot and root) to simulated nitric rain (SNAR) and sulphuric acid rain (SSAR)

T (Days)	Biomass	Cm HNO_3 (pH Concs.)			Cm H_2SO_4 (pH Concs.)			Cm Control
		2.0	3.0	4.0	2.0	3.0	4.0	(6.0)
52	**Leaf g**							
	FW	16.99±0.11[e]	17.79±0.43[b]	18.85±0.13[d]	16.91±0.03[d]	17.99±0.03[d]	19.12±0.03[d]	27.48±0.03[e]
	DW	2.03±0.03[b]	2.30±0.13[b]	2.51±0.03[b]	2.01±0.06[b]	2.12±0.03[b]	2.47±0.48[b]	6.01±0.03[a]
52	**Shoot g**							
	FW	17.27±0.03[e]	18.63±0.24[e]	21.00±0.01[e]	16.89±012[d]	18.10±0.52[d]	20.00±0.01[d]	29.10±0.05[f]
	DW	2.99±0.12[b]	3.00±0.02[c]	3.06±0.03[b]	2.94±0.03[b]	2.98±0.01[b]	3.00±0.21[b]	7.34±0.01[b]
52	**Root g**							
	FW	5.05±0.11[c]	5.27±0.06[d]	5.55±0.01[c]	5.00±0.02[c]	5.18±0.01[c]	5.40±0.05[c]	6.01±0.13[c]
	DW	1.08±0.01[a]	.60±0.44[a]	2.07±03[a]	1.02±0.06[a]	1.50±0.01[a]	2.01±0.02[a]	5.98±0.01[d]

- *Legend is similar to that in Table 1.*

Table 7. Responses of *Amaranthus hybridus* relative growth rate (RGR) to simulated nitric and sulphuric acid rain (SNAR and SSAR)

T (Days)	$gg^{-1}day^{-1}$ HNO_3 (pH Concs.)			$gg^{-1}day^{-1}$ H_2SO_4 (pH Concs.)			$gg^{-1}day^{-1}$ Control
	2.0	3.0	4.0	2.0	3.0	4.0	(6.0)
14	50×10^{-4}±0.1[d]	60×10^{-4}±0.6[d]	69×10^{-4}±0.21[d]	49×10^{-4}±1.3[d]	54×10^{-4}±0.18[d]	63×10^{-4}±0.3[d]	80×10^{-4}±2.6[d]
28	41×10^{-4}±2.1[c]	50×10^{-4}±1.18[c]	59×10^{-4}±1.12[c]	40×10^{-4}±0.8[c]	50×10^{-4}±0.6[c]	55×10^{-4}±1.2[c]	70×10^{-4}±2.9[c]
42	30×10^{-4}±0.15[b]	42×10^{-4}±1.9[b]	50×10^{-4}±1.7[b]	30×10^{-4}±1.7[b]	45×10^{-4}±0.11[b]	49×10^{-4}±2.1[b]	61×10^{-4}±1.9[b]
56	26×10^{-4}±0.4[a]	35×10^{-4}±0.1[a]	39×10^{-4}±2.2[a]	25×10^{-4}±1.6[a]	34×10^{-4}±1.2[a]	38×10^{-4}±1.2[a]	50×10^{-4}±1.7[a]

- *Legend is similar to that in Table 1.*

Table 8. Responses of *Amaranthus hybridus* chlorophyll (Chl.) content to simulated nitric rain (SNAR) and sulphuric acid rain (SSAR)

T (Days)	Chl. type	mg g^{-1} FW HNO$_3$ (pH Concs.)			mg g^{-1} FW H$_2$SO$_4$ (pH Concs.)			mg g^{-1} FW Control
		2.0	3.0	4.0	2.0	3.0	4.0	(6.0)
	Chl. a	24.1±0.3[d]	29.5±0.2[d]	37.6±0.06[d]	24.0±0.43[d]	27.6±0.03[d]	33.7±0.3[d]	45.4±0.1[a]
14	Chl. b	28.4±0.06[d]	34.6±0.3[d]	44.2±0.3[d]	27.8±0.3[d]	32.5±0.3[d]	39.9±0.3[d]	58.3±0.3[a]
	Chl a+b	52.5[d]	64.1[d]	81.8[d]	51.8[d]	60.1[d]	73.6[d]	103.7[a]
	Chl a/b	0.8	0.9	0.9	0.8	0.8	0.8	0.8
	Chl. a	20.4±0.3[c]	24.6±0.1[c]	28.7±0.3[c]	20.0±0.3[c]	23.6±0.43[c]	26.5±0.3[c]	58.2±0.3[b]
28	Chl. b	25.1±0.6[c]	29.2±0.3[c]	36.4±0.05[c]	23.6±0.3[c]	27.4±0.06[c]	33.1±0.3[c]	71.2±0.3[b]
	Chl a+b	45.5[c]	53.8[c]	65.1[c]	43.4[c]	51.0[c]	59.6[c]	129.4[b]
	Chl a/b	0.8	0.8	0.8	0.8	0.9	0.8	0.8
	Chl. a	18.9±0.12[b]	20.7±0.3[b]	25.0±0.2[b]	18.0±0.03[b]	19.6±0.3[b]	24.6±0.08[b]	65.4±0.05[c]
42	Chl. b	23.4±0.04[b]	25.1±0.12[b]	30.4±0.3[b]	20.9±0.1[b]	24.2±0.3[b]	28.3±0.3[b]	84.6±0.3[c]
	Chl a+b	42.3[b]	45.8[b]	55.4[b]	38.9[b]	43.8[b]	52.9[b]	150.0[c]
	Chl a/b	0.8	0.8	0.8	0.9	0.8	0.8	0.7
	Chl. a	17.8±0.1[a]	19.9±0.43[a]	22.4±0.3[a]	17.0±0.43[a]	18.9±0.3[a]	22.6±0.3[a]	74.8±0.01[d]
56	Chl. b	20.0±0.03[a]	23.0±0.20[a]	26.9±0.3[a]	19.7±0.3[a]	22.8±0.3[a]	25.1±0.16[a]	89.1±0.03[d]
	Chl a+b	37.8[a]	42.9[a]	49.3[a]	36.7[a]	41.7[a]	47.7[a]	163.9[d]
	Chl a/b	0.9	0.9	0.8	0.9	0.8	0.9	0.8

- *Legend is similar to that in Table 1.*
- *Chl a = Chlorophyll a, Chl b = Chlorophyll b, Chla+b = Total chlorophyll, Chl a/b = Ratio of chlorophyll a:b.*

Table 9. Observable morphological responses of *Amaranthus hybridus* L. to simulated nitric and sulphuric acid rain (SNAR and SSAR)

pH concentrations	Observed effects
6.0 (Control)	The plants had their normal growth and were healthy
4.0	Mild chlorosis, the plant had good but reduced growth
3.0	Moderate leaf chlorosis and deformation and stunted growth
2.0	Necrosis, severe leaf chlorosis and deformation with severely stunted growth

Plant biomass responses were substantially reduced by acidity of SNAR and SSAR. The study revealed a highly significance difference in FW and DW of leaves, stems and roots between acids rain treated plants compared to the control plants. This confirms the stressful effects of acid rain on the vegetative growth and plant biomass similar to the findings of [20]. Decrease in biomass is in agreement to the finding of [21]. The growth of the plant was hampered due to increase in acidity leading to a decrease in biomass [22]. Another study by [18] showed that acid rain stress induced significant reduction in stem weight by slowing cell division and expansion. They also reported increased rain water acidity and decreased redistribution of photosynthesis, which affects plant root elongation.

The relative growth rate of *A. hybridus* was also adversely affected by SNAR and SSAR. This has been reported by [23,10]. Relative growth rate gives a critical and adequate growth evaluation about the plant.

Photosynthetic pigments were adversely affected with respect to acidity levels. Chlorophyll a, b and total chlorophyll content were significantly reduced by acids rain treatments when compared to the control. These results are in line with earlier reports by [24] on chlorophyll content reduction. The reduction may be due to magnesium ion removal from the tetrapyrol ring of the chlorophyll molecule by hydrogen ion [25] or due to increase in transpiration by acid rain [26]. Reduction in chlorophyll content may also be ascribed to reduction in leaf area [27]. Similar results have been observed in [28,29]. Reduction in chlorophyll content posed by SNAR and SSAR affects photosynthesis which is the primary source of energy for the plant with attendant reduction in growth. According to [30] acid rain generally weakens plants such that they become more susceptible to damage from insects, disease, drought and forms of environmental stress.

Observable morphological changes in this work were; necrosis, chlorosis and leaf deformation and stunted growth. This is in accordance with the work of [19,28,30,31,32]. Physiological and morphological changes induced by SNAR and SSAR on *A. hybridus* are of keen interest because this vegetable is grown mainly for its leaves. Physiological and morphological processes are linked, changes in one affects the other. Reduction in leaf area posed by acids rain on *A. hybridus* affected its chlorophyll content which will invariably affect photosynthesis, decrease in growth; shoot height and all others growth parameters with attendant effect on yield loss (biomass). *Amaranthus hybridus* is not only a vegetable plant, but is also a very important ornamental plant in the world.

5. CONCLUSION

It is evident from this study that *Amaranthus hybridus* depicted negative responses to simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR) treatments with decrease in growth, chlorophyll content and yield. Findings of this research have shown that simulated nitric and sulphuric acid rain of concentrations (pH 2.0, 3.0 and 4.0) applied separately produced negative effect on the physiological and morphological characteristics of *A. hybridus*. Acid treated plants showed reduced growth with increasing acidity due to a reduction in chlorophyll content. Important preventive as well as control measures should be taken to curtail the effects of acid rain on *A. hybridus* and other crop plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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