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GROWTH AND PHOTOSYNTHETIC RESPONSE OF *Capsicum annum* L. EXPOSED TO SIMULATED ACID RAIN.

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ABSTRACT

Rain is inherently acidic, human anthropogenic activities including pollution from homes, companies, power plants, and car vehicles, which result in the release of molecules like Carbon dioxide, Nitric dioxide, and Sulfur dioxide, are making it more and more acidic. The screen house experiment was undertaken to assess how acid rain affects plants. In the lab, to create acid rain concentrated sulphuric acid (H_2SO_4) and concentrated nitric acid (HNO_3) were mixed in a 2:1 ratio. The desired pH level was measured using a Duplex pH meter. The plant was simulated with acid rain (pH 2.5, 3.0, 3.5, and control 5.6) every three days for six weeks. ANOVA was used for the statistical analysis. The significance of the mean difference was assessed using Ducan's multiple-range tests. With increasing pH levels, plant height ($F_{Pepper} = 63.835$), leaf area ($F_{Pepper} = 60.965$), stem girth ($F_{Pepper} = 39.3$), the number of leaves ($F_{Pepper} = 34.265$), relative growth rate ($F_{Pepper} = 40.646$), and chlorophyll content (F_{Pepper} = 4.6029, 7.8154, 36.746) decreased considerably ($p<0.001$). These findings advance our knowledge of how acid rain impacts urban vegetation and serves as a warning to limit acid rain production.

Keywords: *Capsicum annum***,** simulated Acid rain, Growth, Photosynthesis.

INTRODUCTION

Precipitation that is abnormally acidic and contains significant levels of H+ is referred to as "acid rain" (Lal, 2016). Acid rain is formed when Sulfur Dioxide and Nitrogen Oxide emissions interact with atmospheric water to make atmospheric pollutants. Rainwater's pH drops below 5.6 when acid is added to it (Dondapati *et al.*, 2013). Urban areas and heavily industrialized areas have experienced problems with acid rain (Grennfelt et al., 2020).

The majority of air pollutants are produced worldwide by burning fossil fuels for electricity generation, heating, transportation, and industry (US Environmental Protection Agency, 2017). Though rain is inherently acidic, human anthropogenic activities including pollution from homes, companies, power plants, and car vehicles, which result in the release of molecules like Carbon dioxide, Nitric dioxide, and Sulfur dioxide, are making it more and more acidic (Chandra *et al.,* 2017).

According to reports, acid rain causes anomalies in physiological processes in plants, including photosynthesis, chlorophyll concentration, nitrogen metabolism, and the generation of reactive oxygen species (Martins *et al.,* 2013). Chlorophyll content reduction in a leaf is a sign of direct foliage damage (Sun *et al.,* 2016). As a result, it directly impacts how plants grow and what they can do to facilitate photosynthesis, which is significantly hampered by low pH. Particularly, pH has an impact on the majority

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of plant agro morphological parameters (Jiang *et al.,* 2017). This study aims to evaluate the plant's adaptability in terms of its morphology, and physiology. The research also adds to environmental preservation and sustainable development in regions throughout the world that is affected by acid rain.

MATERIALS AND METHODS

Study Location

The Federal University of Technology Owerri in Imo State served as the study's location. The state can be found in Nigeria's southeast. Between latitudes 4°45'N and 7°15'N and longitudes, 6°50'E and 7°25'E where it is located (NIMET, 2016). The region is in a tropical rainforest zone with the wet season from April to October and the dry season from November to March: The local daytime highs and lows were 18 and 34 °C. The range of the daily average temperature is 19°C to 28°C.

Preparation of Acid Rain

According to Thompson et al. (2016), concentrated sulfuric acid (H_2SO_4) and nitric acid (HNO_3) were combined in a 2:1 ratio to create acid rain. The appropriate pH levels (2.5, 3.0, 3.5, and control 5.6) were created using a pH meter and distilled water.

Experimental Design and Treatments

In experimental pots, five seeds of each plant were planted, and each pot received 10 ml of an acidic solution per the pH values of 2.5, 3.0, 3.5, and the control (5.6). In a completely randomized design, there are three replicates for each treatment (CRD). Using a medium-sized pressurized sprayer, the solutions were administered once every three days. Six weeks of plant growth followed before the experiment came to an end.

Extraction of Chlorophyll

According to a procedure published by Harborne in 1987, 1gm of freshly cut leaves was ground using a pistol and mortar and 20–40ml of 80% acetone. For five minutes, the solution was centrifuged at 5000–10,000 rpm. The operation was repeated until the colorless residue was produced, then the supernatant was transferred to another test tube. Using a device spectrophotometer, the solution's absorbance was measured at 645 and 663 nm in comparison to a solvent (acetone) blank.

Measurement of Chlorophyll

Using a spectrophotometric approach, the amount of chlorophyll-a and b in the extract chlorophyll was measured. Chlorophyll absorbance was measured at 645 nm and 663 nm, respectively. To get a value that was preferably in the range of 0.2 to 0.8 absorbance units, the extract was diluted with 80% acetone. The equation below was used to compute the concentrations of total chlorophyll, chlorophyll-a, and chlorophyll-b in the sample:

Total Chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a: $12.7(A_{663}) – 2.69(A_{645})$

Chlorophyll b: $22.9(A_{645}) – 4.68(A_{663})$

Note:

A_{645} = absorbance at a wavelength of 645 nm;

A_{663} = absorbance at a wavelength of 663 nm.

Height of the Shoot

Using a tape rule, the shoots' height was measured in (cm), from the soil's surface to the plant's apex. The measurements were taken at intervals of 14 days after the start of the simulated acid rain treatment.

Number of Leaves and the Area

The number of leaves per plant was manually counted and recorded. Leaf area (cm^2) was calculated by measuring the length of the leaf from base to tip and the width from the widest part of the leaf blade. The leaf area was calculated by multiplying the length by the breadth and the leaf correction factor by 0.578 (Masarirambi *et al.,* 2012).

Girth Length

The measurement of the girth (cm^2) was made using a vernier caliper.

Relative growth

Following the procedures of (Hunt 1990), the fresh weights and the Relative Growth Rate (RGR) were computed. The relative growth rate was calculated using the fresh weight of the entire plant:

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

Note:

W_2 = weight of plant at end of the experiment

W_1 = weight of plant at the interval (2 weeks)

T_2 =final time

T_1 = initial time.

Statistical Analysis

A one-way analysis of variance approach was used to statistically analyze the experimental data. The R 4.2.1 software program was used for all analyses. Ducan's multiple-range tests were used to assess the differences between means that were statistically significant ($p<0.001$). The means of ten experimental replications from each treatment comprised all the results that were published.

RESULTS

Measurement of Growth Parameters

The result (table 1) shows the growth of plant in term of height, leaf area, stem girth leaf count and relative growth rate were reduced significantly ($p<0.001$) as the pH level increased. Minimum growth was observed at pH2.5 while maximum growth was seen at the control (pH 5.6).

Measurement of Chlorophyll Content

The result (table 2) shows decreased in chlorophyll content significantly ($p<0.001$) when the pH level increased. Low chlorophyll content (total chlorophyll) was measured (43.6342 mg/g) when the pH is at 2.5 while higher (71.0202 mg/g) at the control.

Table 1: Plant height, leaf area, Stem girth, Number of leaves, and RGR of *C. annum*

pH level	Plant Height (cm)	Leaf area (cm^2)	Stem girth (cm)	Number of leaves	RGR ($gg^{-1}d^{-1}$)
pH2.5	16.81^d	5.177^d	1.06^d	32.6^d	0.333^d
pH3.0	22.77^c	9.138^c	1.50^c	47.7^c	0.532^c
pH3.5	27.53^b	12.999^b	1.90^b	66.6^b	0.708^b
pH5.6 (Control)	34.83^a	16.838^a	2.37^a	79.5^a	0.974^a

*Mean value Followed by the same latter in a column of each parameter shows no significance different at ($p<0.001$)

Table 2: Chlorophyll Content of *Capsicum annum*

pH level	Chlorophyll (mg/g)		
	Chl A	Chl B	Chl AB
pH2.5	17.3718^b	26.2774^c	43.6342^d
pH3.0	23.8860^a	33.4422^{bc}	57.3090^c
pH3.5	25.8624^a	37.4508^{ab}	63.2916^b
pH5.6 (Control)	26.4412^a	44.5956^a	71.0202^a

*Mean value Followed by the same latter in a column of each parameter shows no significance different at ($p<0.001$)

DISCUSSION

The results demonstrated that the control (pH5.6) exhibits the maximum performance in height, leaf area, stem girth, and leaf number significantly ($p<0.001$) when compared to other acidic treatments. Low pH causes a reduction in stem and girth length, which is regarded as slower growth (Wang, 2010). Low pH inhibits plant development and changes plasma membrane function by concentrating Al^{3+} to hazardous levels. Although leaf area increases the plant's dry mass, it does not significantly boost photosynthesis (Li et al., 2019). The results of Pharm et al. (2021), Vina et al. (2020), Han (2019), Liu et al. (2018), and Rani (2017) are in agreement with those of our study.

Chlorophyll levels decrease with increasing acidity, as shown in (Table 2). The control (pH5.6) exhibits substantially more chlorophyll than other treatments ($p<0.001$). Reduced leaf area cause decrease in photosynthesis and chlorophyll concentrations (Li et al., 2019). When exposed to acid rain, chloroplast growth and chlorophyll production were similarly significantly impacted. Although it is inconsistent with the invisible or anatomical damage at pH 3.5, this most likely represents the substantial damage seen at pH 2.5. This suggests that physiological harm may exist even in the absence of morphological or anatomical damage. Similar findings have been made by Vina et al., Long et al., (2017) Kausar et al., (2019) Shaukat et al., (2018), and Vina et al., (2020).

CONCLUSION

Plants treated with simulated acid rain showed visible morphological damage, indicating that morphological damage led to anatomical damage. Simulated acid rain with pH below 3.5 shows anatomical damage, but the damage was more severe below pH 2.5. Even at pH 3.5, where apparent damage was not present, acid rain decreased the amount of chlorophyll a and b, suggesting that physiological damage resulted from anatomical damage. In comparison to the control, all treatments reduced the growth parameters including plant height, leaf area, stem girth, relative growth rate, and leaf count.

These findings were made under controlled conditions in a screen house, but the impacts of acid rain in actual metropolitan settings may differ and need to be addressed. Overall, our research helps us better understand how acid rain impacts urban vegetation and raises concerns about the need to curtail its production in urban areas. Additionally, it prompts concern over more effective ways to track how acid rain affects vegetation because it cannot simply be recognized by looking at obvious leaf damage.

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