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Simulated acid rain impact on growth, yield and leaf anatomy of *Dioscorea rotundata* (L.)

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Abstract. *Acid rain causes damage to crops and also has adverse effect on the soil and the environment. These have led to the abandonment of farmlands in affected areas. Southern Nigeria which is known for yam cultivation is subjected to acid rain resulting from gas flaring activities, importation of fairly used vehicles, power generation and industrial activities. The growth, yield and leaf anatomy response of Dioscorea rotundata (L.) exposed to simulated acid rain (SAR) of pH 2.0, 3.0, 4.0 and 6.0 (control) was investigated. Acid rain exposure caused morphological changes in plants including chlorosis, necrosis, and leaf folding. Acidic precipitation inhibited vine length, leaf number, chlorophyll content index and leaf area at pH 2.0; while growth was encouraged at pH 6.0. 22% of leaf surface area of D. rotundata L, was injured after simulated acid rain treatment at pH 2.0 while necrosis was absent at the (control) pH 6.0. Harvest index of D. rotundata was not significantly different (p>0.05) between the pH treatment groups; however, was slightly higher at the (control) pH 6.0. Percentage dry matter partitioning of D. rotundata leaf and stem was not affected by the acid rain exposure; nevertheless, maximum percentage dry matter of tuber was obtained at pH 4.0 and pH 6.0. Transverse sections of D. rotundata leaves showed cuticle wax damage and rupture of epidermis at lower pH 2.0 and 3.0. Mesophyll degradation and cytoplasm depletion was also observed in the treated leaves. The plant leaves revealed dark tissues in necrotic areas which may be phenolic compounds secretion. However, it became clear that significant increases in the growth parameters considered occurred at pH 6.0 (control).*

Keywords: chlorosis, morphological parameters, necrosis, simulated acid rain, structural changes, tuber

Introduction

Rain water in its natural state is slightly acidic with a pH value ranging from 5.6-7.0. This is due to the dissolution of atmospheric carbon dioxide (Nduka et al., 2008). Acid rain is the decrease in pH of rain water resulting from human activity. It results from the burning of fossil fuels, releasing NO_x and SO_x (Singh and Agrawal, 2008; Abasi et al., 2013). When these pollutants dissolve in rain water they form nitric acid and sulphuric acid. Atmospheric SO_2 has increased twenty fold globally since the 1800s (Lehmann et al., 2008). When acid precipitation falls, it can affect forest, fields, gardens and aquatic plants. It can also affect paintings on buildings, eroding limestone structures and sculptures, kills or dwarfs trees and reduces food crop yields (Knittel and Pell, 1991; Kidd and Kidd, 2006; Ma et al., 2019). Acid rain exposure of plants results in characteristic foliar injury symptoms, modified leaf anatomy structural changes in the photosynthetic pigment apparatus and a decrease in the chlorophyll concentrations (Stoynova and Velikova, 2004; Odiyi and Bamidele, 2014). Sant' Anna-Santos et al. (2006) have reported reduction in plant growth and yield of field corn.

Dioscorea rotundata (L.) belongs to the family of Dioscoreaceae. It is an important tuber crop of the tropics and some other countries in East Asia, South America and India (Iwueke et al., 2003). Yam is among the oldest recorded food crops and ranks second after cassava in the study of carbohydrates in West Africa (Agwu and Alu, 2005). The tubers are eaten boiled, roasted, fried and pounded into yam flour. Yam is one of the major staple foods in Nigeria and has potential for livestock feed and industrial starch production (Anyanwuyi et al., 2011). It is one of the principal tuber crops in Nigeria in terms of land under cultivation and in the volume and value of production (Bamire and Amujoyegbe, 2005).

Southern Nigeria which is known for yam cultivation is subjected to acid rain from gas flaring activities, importation of fairly used vehicles, electricity generation and industrial activities. Efe (2006) reported that gas flaring waste incinerating, bush burning, fumes from fairly used cars, fumes from generators and other anthropogenic activities are the major causes of acid rain in Nigeria. Acid rain has been observed in Warri, the rural area of Delta state, Nigeria and the Niger Delta region of Nigeria (Efe, 2005, 2006, 2011). Considering the importance of *Dioscorea rotundata* in human diet and the adverse effect of acid rain, the study was carried out to investigate the impact of simulated acid rain on this plant species.

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Material and methods

Experimental setup and procedure

A plot of land in the Botanical Garden of the Faculty of Life Sciences, University of Benin, Nigeria, was used for the study. Healthy Ame variety of *D. rotundata* seeds were collected from National Root Crop Research Institute (NRCRI) Umudike, Abia State, Nigeria. Seeds measuring 16 cm long were planted line to line distance 100 cm apart. The depth of the yam seeds from the topmost soil was 12 cm. Yam seeds were planted in four replicates in a complete randomized design (CRD). A transparent roof was built above the plants to prevent natural rain. Yam heaps were watered two times weekly and treatment commenced six weeks after planting. Simulated acid rain (SAR) was sprayed with a 2 liter pressurized plant sprayer from the apex to the lower leaves of the plant at a frequency of one rain treatment per day (50 ml) at 2.5 mm per drop of the rain. During the course of the experiment plants were exposed to simulated acid rain thrice a week. The plants were treated for 21 weeks before the termination of the experiment. The temperature of the experimental site during the period of the experiment was between 24 and 29°C.

Preparation of simulated acid rain

The simulated acid rain was prepared by mixing tetraoxosulphate (VI) acid (H_2SO_4) and trioxonitrate (V) acid (HNO_3) in the ratio 2:1 in distilled water. A Digital pH meter was used to verify the pH of the different acidic water. Sodium hydroxide (NaOH) was used to increase pH or decrease pH when necessary and made up to pH 2.0, 3.0, 4.0 and 6.0 (control).

Determination of growth parameters

Vine length was measured using measuring tape in (cm) from base of plant to the terminal bud. Leaf number was determined by counting the number of leaves of the plant. Leaf area (LA, cm^2) was measured according to the method of Agueguia (1993):

LA (cm^2) = (L x W). K,

Where: K = Constant = 0.927; L = Leaf length (cm), W = Leaf width (cm).

Chlorophyll concentration index (CCI) of leaves was determined using a chlorophyll content meter (CCM 200 plus, Apple G Instrument) to clip yam leaves and chlorophyll concentration read for the different pH treatments. The percentage of leaf surface area necrosis was assessed visually using grid intersections by placing a 1 mm^2 metal net on four leaves taken from different parts of each plant, the mean leaf area taken. The percent of leaf areas injured was defined as the number of 1 mm^2 covering injured areas divided by the total number of 1 mm^2 on the leaf multiplied by 100. This method was in accordance with Cumpertz et al. (1982).

Lesion area (%) = (Leaf spot area / Total leaf area) x 100

Fresh and dry weight was determined after termination of the experiment following the methods of Hunt (1990). Harvest index (HI) was calculated in accordance with Evans (1993):

HI = Economic yield / Total biological yield

Anatomical procedure

Foliar materials for epidermal studies were collected fresh from plants growing in the garden. One centimeter square (1 cm^2) leaf cuttings were obtained from fresh leaf, generally from mid-way between the leaf base and apex of lamina. Fresh samples (leaf margin, lamina and midrib) of *D. rotundata* leaves were fixed in formalin, acetic acid and alcohol (FAA) for 12 hours, dehydrated in alcohol series (30%, 50% 70%, 95% and absolute alcohol) for 3 hours each, cleared in chloroform-alcohol series (3:1; 1:1; 1:3) v/v for 10 minutes in each, wax embedded and sectioned. Good preparations were mounted on slides, viewed and photographed using light microscope with camera attached, model: Optika B-1000 FI Led.

Statistical analysis

The results obtained were subjected to statistical analysis using package for social sciences, version 16.0 (SPSS). Statistical significance between the different groups was determined by Analysis of variance (ANOVA).

Results

Leaf is the most sensitive plant organ to acid rain. Simulated acid rain (SAR) caused serious damage to the yam leaves investigated, lesions were observed after second acid rain spray. Subsequent acid rain precipitation resulted in chlorosis and necrosis which appeared as yellow and brown spots on the leaf surfaces. Necrosis was observed on the leaves sprayed with SAR pH 2.0, pH 3.0, few at pH 4.0 and absent at pH 6.0 (control). The result of the experiment (Table 1) revealed that the acid rain treatment suppressed growth parameters such as vine length and leaf number significantly ($p<0.01$) relative to the control. Chlorophyll content index was significantly inhibited ($p<0.05$) at pH 2.0 compared to the pH 6.0 (control), pH 4.0 and pH 3.0.

Table 1. Effects of simulated acid rain (SAR) on vine length, leaf number and chlorophyll content index (CCI) of *D. rotundata* (n=4)

Growth parameters	pH of SAR				p - value
	6.0	4.0	3.0	2.0	
Vine length (cm)	946.50±31.81[a]	833.50±47.02[b]	716.50±42.60[c]	596.25±62.22[d]	p<0.01
Leaf number	859.25±54.34[a]	717.50±32.49[b]	619.50±53.27[c]	562.00±38.59[d]	p<0.01
CCI	31.575±1.748[a]	31.850±1.773[a]	30.150±2.225[a]	23.250±1.541[b]	p<0.05

*CCI- Chlorophyll concentration index; Each value is a mean ± standard error of four replicates; All similar alphabets show means that are not significantly different.

Table 2 the data show the leaf area decrease from the control treatment (pH 6.0) to the simulated acid rains treatments – by 28.8% at pH 4.0, by 34.4% at pH 3.0 and by 40.3% by pH 2.0. The differences in the leaf area between all treatments were statistically significant ($p<0.05$). Harvest index values at control group (pH 6.0) were higher than those of the treatment groups but the differences were not statistically significant ($p>0.05$). Percentage leaf surface area with necrosis decreased with increase in pH at the simulated acid rain precipitation from 3% at pH 4.0 to 22% at pH 2.0 while necrosis was absent at the pH 6.0 (control).

Table 2. Effects of simulated acid rain on leaf area, harvest index and percentage necrotic area of *D. rotundata* (n=4)

Growth Parameters	pH				p- value
	6.0	4.0	3.0	2.0	
Leaf area (cm^2)	55.28±7.64^a	42.65±5.42^b	36.24±4.51^c	33.02.±5.44^d	($p<0.05$)
Harvest Index	0.660±0.03^a	0.658±0.055^a	0.555±0.053^a	0.608±0.031^a	($p>0.05$)
Necrotic area (%)	0	3	16	22	

*Each value is a mean ± standard error of four replicates; Note: All similar alphabets show means that are not significantly different.

The experimental result (Figure 1) demonstrates that the percentage of the leaf (15.41-18.90%), stems (16.30-23.62%) and tubers (57.48-67.30%) dry weight of *Dioscorea rotundata* were not significantly affected by simulated acid rain. Tubers dry weight was a little higher at pH 4.0 and the control pH 6.0 compared at pH 2.0 and 3.0.

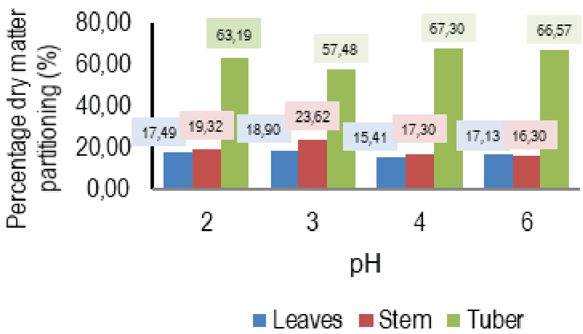

Figure 1. Percentage dry matter partitioning of *D. rotundata* after simulated acid rain exposure

Effects of simulated acid rain on leaf anatomy of Dioscorea rotundata

This study investigated the effects of simulated acid precipitation on leaf anatomy (Figure 2). Visible leaf injury was observed when *D. rotundata* was sprayed with acidic rain at (pH 2.0 and 3.0). Simulated acid rain with higher pH (control, pH 6.0) was not injurious and did not result in visible leaf damage. Treatment with a solution of pH 4.0 relative to the control showed scanty and tiny necrotic spots and therefore was not included in the figure displayed. Acidic precipitation caused veinal and in between the veins necrotic spots with dark appearance. These symptoms occurred after the second rain application both on the adaxial and abaxial leaf surface. The spots number increased and became wider as the experiment progressed. Transverse section of *D. rotundata* leaf at pH 6.0 (control) showed healthy adaxial epidermis and mesophyll consisting of palisade parenchyma and spongy parenchyma followed by the abaxial epidermis (Figure 2-A). The injuries caused by SAR at pH 3.0 (Figure 2-B) revealed the presence of brown spots which resulted in collapse of leaf tissues in the affected area. At pH 2.0 (Figure 2-C), the cells near the necrotic area were ruptured and showed distortions in the arrangement of the palisade and spongy mesophyll. In the pH 6.0 (control) treatment, features of the leaf margin (Figure 2-D) revealed the presence of turgid epidermal cells with distinct contours. SAR treated leaf margin at pH 3.0 showed distorted epidermal and mesophyll cells marking the beginning of necrosis (Figure 2-E). At pH 2.0 (Figure 2-F), leaf margin collapsed with dark tissues which might be as a result of secretion of phenolic compounds. Leaf mid-rib at pH 6.0 (control) revealed healthy tissue with intact and well defined cell shape (Figure 2-G). SAR caused necrotic mid-rib at pH 3.0 (Figure 2-H); the mesophyll layer was disoriented with ruptured cells. The tissues were collapsed on the necrotic mid-rib (Figure 2-I) and the cell contents were not recognized.

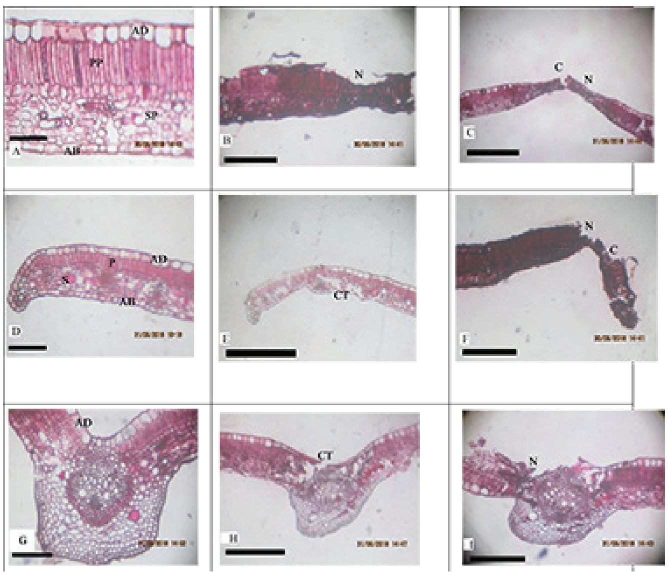

Figure 2. Leaf transverse section of *D. rotundata*: (A, D, G)- control treatment (pH 6.0); (B, E, H)- acid rain pH 3.0; (C, F, I)- acid rain pH 2.0; (A) Lamina mid- region (330 µm); (B) Necrotic lamina (320 µm); (C) Area between the affected and healthy lamina (630 µm); (D) Healthy leaf margin (430 µm); (E) Beginning of marginal necrosis (470µm); (F) Marginal necrosis (430 µm); (G) Mid- rib region (120 µm); (H) Ruptured mid- rib (130 µm); (I) Collapsed and necrotic areas (130µm); ADE- Adaxial epidermis; ABE- Abaxial epidermis, PP- Palisade parenchyma; SP- Spongy Parenchyma; N- Necrotic areas; CT- collapsed tissue.

Discussion

The results obtained from this study showed that SAR affected all the growth parameters measured of *D. rotundata*. Lesions were formed after the second acid rain precipitation. Once a lesion is formed, it acts as a depression for collection of rainfall. The enlargement of these lesions is probably accelerated by the pool formed by subsequent rainfalls. Visual symptoms observed on *D. rotundata* leaves were chlorosis, necrosis and leaf folding. Simulated acid rain used in this study caused necrosis and chlorosis at the lower pH 2.0 and 3.0, slightly at pH 4.0 and absent at the control pH 6.0. Rodriguez-Sanchez et al. (2020) reported that leaves of *Liquidambar styraciflua* displayed brown spots when sprayed with simulated acid rain of pH 2.5. These findings were similar to the work of Odiyi and Bamidele (2014) who established that leaves of *Manihot escunlenta* exposed to simulated acid rain showed chlorosis and necrosis. Acid rain precipitation reduced vine length and leaf number significantly at pH 2.0 relative to the control. According to Dursun et al. (2002), simulated acid rain inhibited shoot growth of tomato (*Lycopersicon esculentum*) with the strongest inhibition recorded at pH 2.5. In a study on *Solanum lycopersicum* L., Eguagie (2015) found that SAR induced reduction in number of leaves. Leaf area was reduced at pH 2.0 by 40.3% as compared to the control (pH 6.0), which is statistically significant ($p<0.05$), (Table 2). Singh and Agrawal (2004) reported that leaf area of two wheat cultivars M213 and Sonalika declined at pH 4.0 and 3.0 when subjected to simulated acid rain. Chlorophyll content index decreased significantly at pH 2.0 as a result of acidic precipitation relative to the control. Lal and Singh (2017) observed that acid rain caused a marked decline in chl-*a* chl-*b* and carotenoids in sunflower leaves at peak growth stage. Percentage leaf surface area necrosis decreased with decrease in simulated acid rain concentration. Mai et al. (2008) observed visible injury, decline in leaf area and mass of fresh leaf per unit area of winter wheat cv. at pH 2.5. Odiyi and Eniola (2015) reported chlorosis and necrosis in leaves treated with simulated acid rain of pH 2.0.

Harvest index of *D. rotundata* tubers was slightly affected by the various pH levels of simulated acid rain treatment. Iglesias et al. (1994) reported that harvest index of 0.5-0.6 is the optimum level because at higher values of harvest index, root production decreases due to reduced leaf area, light interception and photosynthesis. In our study percentage dry matter partitioning of leaf and stem was not affected by simulated acid rain. Percentage tuber dry weights decreased slightly at the lower pH relative to the control. Chung et al. (1994) reported that leaf dry weight and root dry weight of *Perilla frutescens* were significantly reduced at pH 2.5. Kauser et al. (2010) established that simulated acid rain suppressed dry weight of shoot and root in wheat cv. HD-2329 at pH 3.0. Lal and Singh (2012, 2015) studied the effects of simulated acid rain (pH 7.0 - control, 5.7, 4.5, and 3.0) on plant root, shoot, and leaf in sunflower *(Helianthus annuus)* cv. Their results revealed that biomass and lengths of the studied plant parts decreased with decreasing pH of acid rain solution. Visible leaf injury was observed when *D. rotundata* was sprayed with acidic rain at pH 2.0 and 3.0. Transverse sections of the leaf lamina, margin and midrib investigated at pH 6.0 revealed healthy tissues with well-defined cell shape. Acid rain treated leaves at pH 2.0 and 3.0 caused collapse in epidermis exposing the parenchyma cells to severe damages. The anatomical analysis of injuries caused by pollutants on plant species has been used in various studies to access the real damage caused by pollutants (Silva et al., 2005; Medeiros et al., 2017; Carvalho-Andrade et al., 2020). Acid solution deposited on leaves affect the epidermal cells causing cuticle damage and altering the leaf permeability (Dan et al., 2017). In *D. rotundata* necrosis was located in the veins and in between the veins as a result of rainwater collecting in these regions. Lal et al. (2017) reported that necrotic symptoms first appeared along the veins and spread gradually into the interveinal areas when leaf of sunflower (*H. annuus*) was treated with SAR of pH 3.0. The cells near the necrotic area in this study, showed changes in the arrangement of the mesophyll. The structures of the epidermal and mesophyll cells could not be identified but healthy cells occurred adjacent to collapsed necrotic area (Silva et al., 2005). Dark contents which may be phenolic compounds secretion were noticed in the SAR treated tissues. Zobel and Nighswander (1991) reported that the accumulation of these compounds was generally followed by cytoplasm degradation and vacuolar content release that led to cell death. The secretion of phenolic substances signifies stress in the acid rain treated leaves. SAR at lower pH is likely to cause stomata to be ruptured exposing the more internal plant tissues to direct effects of acid rain, as observed in *D. rotundata.*

Conclusion

Simulated acid rain - SAR (pH 2.0-4.0) exposure caused morphological changes in *Dioscorea rotundata* including chlorosis, necrosis, and leaf folding. SAR treatment at pH 2.0 and pH 3.0 has inhibitory effect on growth, yield and leaf anatomy of *D. rotundata.* Visual assessment alone was not sufficient to determine the real effects of SAR, hence the use of leaf anatomy for determination of necrosis. The occurrence of cellular injury using microscopic view revealed that anatomical investigations showed distortions in the adaxial and abaxial epidermis, palisade parenchyma and spongy parenchyma resulting in a decline in growth. In view of growing industrialization, there is need to observe the effects of acid precipitation on related species.

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