Biological and Environmental Sciences Journal for the Tropics
Vol. 21, Number 2, August 2024. Pp 9 - 14
https://dx.doi.org/10.4314/bestj.v21i2.2
Research article

Simulated acid rain caused damage on leaf anatomy reduce growth and yield of *Dioscorea alata* (L.)

Edosa, E. P*, Bamidele, J. F

Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, P.M.B 1154, Benin City, Edo State Nigeria.

*edosaehiaghe@gmail.com

Submission: 02/03/2024
Accepted: 06/08/2024

Abstract
Most crops grown in the Southern part of Nigeria are exposed to acid rain owing to the release of sulphur and nitrogen oxides into the atmosphere. These gases emanate from man's activities such as vehicular movements, electricity generation gas flaring and industrial activities. A mixture of sulphur oxides, nitrogen oxides and atmospheric water or water vapour gives rise to acid rain. This study was undertaken to determine the influence of simulated acid rain on the growth, yield and leaf anatomy of *Dioscorea alata*, water yam. The plants were exposed to simulated acid rain of pH 2.0, 3.0, 4.0 and 6.0 (control) respectively. Necrosis and chlorosis were observed on the yam leaves, after simulated acid precipitation. The impact of simulated acid rain became more noticeable with increase in acidity. Vine length, leaf number, leaf area and chlorophyll content index were inhibited ($p<0.05$) at pH2.0 and pH3.0 as compared with the control. At pH2.0, 28% leaf surface area of *D. alata* was injured after simulated acid rain treatment while necrosis was absent at pH6.0. Acidic precipitation did not significantly ($p>0.05$) affect harvest index in *D. alata*, however, highest value was found at pH6.0. Anatomical sections of *D. alata* leaves revealed cuticle wax damage and breakage of epidermis at lower pH2.0 and 3.0. Mesophyll tissue breakdown was also observed in the treated leaves.

Keywords: Chlorosis, *Dioscorea alata,* growth parameters, necrosis, simulated acid

Introduction
Acid rain has become an environmental problem universally in the last decades. It can have deleterious effects on plants, aquatic animals and infrastructure. Acid rain is caused by emissions of sulphur oxide and nitrogen oxide, which react with the water molecules in the atmosphere to produce acids (Abbasi et al., 2013; Edosa and Bamidele, 2021). Acid rain is the deposition of wet (rain, snow, fog, dew and cloud water) and dry acid molecules and gaseous acid components (Weathers and Likens, 2006). Acid rain can cause damage to forest, aquatic plants, fields and gardens, affect paintings on buildings, eroding limestone structures and sculptures, kills or dwarf trees and reduces food crop yields (Kidd and Kidd, 2006; Ma et al., 2019). Acid rain exposure of plants results in foliar injury symptoms, alteration in leaf anatomy, structural changes in the photosynthetic pigment apparatus and a decrease in the chlorophyll content (Silva et al., 2005b); injures the waxy layer on the leaves and makes them vulnerable to diseases (Banwart *et al*., 1990). Acid rain has negative effect on seed germination of rice, wheat, grapes and prevents reproduction of these plants (Huang *et al*., 2005). Yam is one of the necessary food in Nigeria and has potential for livestock feed and industrial starch production (Anyanwuyi *et al.*, 2011). Water yam *Dioscorea alata* (L.) which belongs to the family Dioscoreaceae was used in this study. According to Wireko *et al.,* (2013), water yam contains high level of total dietary fibre (TDF) which makes it suitable for management of pile, constipation and diabetes. It is also rich in vitamin C, Beta carotene, vitamin E, calcium, potassium, magnesium,

copper and antioxidants. These nutrients are known to play vital role in general body upkeep as well as immune functioning wound healing, suppression of blood sugar, bone growth and anti-ageing. Yam cultivated in the Southern part of Nigeria is exposed to acid rain from vehicular emissions, electricity generation, industrial discharges and gas flaring activities. Acid rain has been observed in Warri the rural areas of Delta State, and the Niger Delta region of Nigeria (Efe, 2005). Efe (2010) reported that gas flaring, waste incineration, bush burning, fumes from fairly used cars, fumes from generators and other anthropogenic activities are the major causes of acid rain in Nigeria. Considering the importance of this plant in human diet and the detrimental effect of acid rain, the study was carried out to examine the impact of simulated acid rain on the leaf anatomy, growth and yield of water yam plant.

Materials and Methods

Experimental setup and procedure

A plot of land measuring 5 m x 6 m inside Botanical Garden, Faculty of Life Science, University of Benin, Nigeria, was used for the study. Healthy 98/01166 variety of *D. alata* seeds were collected from National Root Crop Research Institute (NRCRI) Umudike, Abia State, Nigeria. Seeds measuring 18 cm long were planted line to line distance 100cm 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 was sprayed with a 2 liter pressurized plant sprayer from the apex to the lower leaves of the plant at a frequency of three rain treatments per week at 2.5 mm per drop of the rain. The plants were treated for twenty-one weeks before the termination of the experiment. The temperature of the experimental site during the period of the experiment was between (24° - 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 decrease pH when necessary and made up to 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 was measured according to the method of Agueguia (1993).

LA = (L X W) K

Where K = constant = 0.927

L = Leaf length, W = Leaf width

Chlorophyll concentration index (CCI) of leaves was determined using a chlorophyll content meter (CCM 200 plus, Apogee Instrument) to clip yam leaves and chlorophyll concentration read for the different pH treatments. The percentage leaf surface area necrosis were assessed visually using grid intersections by placing a 1mm2 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 1mm2 covering injured areas divided by the total number of 1mm2on the leaf multiplied by 100. This method was in accordance with Cumpertz et al., (1982).

Percentage lesion area = Leaf spot area / Total leaf area x 100

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

H.I = Economic yield / Total biological yield

Anatomical Procedure

Foliar materials for epidermal studies were collected fresh from plants growing in the garden. One centimeter (1cm) square 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.alata* 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 sections were mounted on slides, viewed and photographed using light microscope with camera attached, model: Optika B-1000 Fl Led.

Data Analysis

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

Results

Leaf damages were first noticed after the second simulated acid rain exposure. Growth in vine length, leaf number, leaf area and chlorophyll content index (Table 1) was significantly decreased ($p<0.05$) by simulated acid rain at pH2.0 compared to the (control) pH6.0. Harvest index of *D. alata* (Table 1) was not significantly ($p>0.05$) affected by simulated acid rain; nevertheless, highest value was recorded at the control. Leaf surface area with necrosis (Table 1) decreased with increase in pH of simulated acid rain. Simulated acid rain at pH2.0 damaged 28% leaf surface area; 20% at pH4.0; 4% at pH3.0 while necrosis was absent in the (control) pH6.0.

Table 1: Impact of simulated acid rain (SAR) on vine length (cm), leaf number, leaf area (cm^2), chlorophyll content index, harvest index and percent necrotic area of *Dioscorea alata* 21 weeks after treatment**.**

Growth	pH of SAR				
Parameters	6.0	4.0	3.0	2.0	P - value
Vine length (cm)	977.00±21.381^a	861.00±73.000^b	763.00±56.920^c	701.00±62.098^c	P<0.05
Leaf number	541.75±48.810^a	403.00±31.292^b	386.00±28.309^c	347.00±25.547^c	P<0.05
Leaf area	173.50±18.5^a	143.25±11.3^b	104.75±9.6^c	88.75±11.6^c	P<0.05
Chlorophyll content index	34.150±0.750^a	29.850±2.736^b	23.350±2.247^c	24.025±1.707^c	P<0.05
Harvest index	0.618±0.067^a	0.575±0.025^a	0.503±0.063^a	0.530±0.028^a	
Percent necrotic area (%)	0	4	20	28	P>0.05

Each value is a mean ± standard error of four replicates. All similar alphabets shows mean that are not significantly different

Leaf anatomy of *Dioscorea alata*

The leaves of *Dioscorea alata* when subjected to simulated acid rain revealed injuries that appeared as whitish spots initially but later turned brownish. Leaf sections including leaf lamina, margin and midrib were taken from the (control) pH6.0 and the lower pH2.0 and 3.0. The transverse section of *Dioscorea alata* leaves at pH6.0 showed healthy adaxial epidermis, mesophyll and abaxial epidermis (Figure 1A). Treatment with a solution of pH4.0 relative to the control showed scanty and tiny necrotic spots. Acidic precipitation caused necrotic spots in the veins. The injuries caused by SAR at pH3.0 (Figure 1B) showed shrinking of the epidermis and mesophyll in affected areas. The epidermal and parenchyma cells at pH2.0 (Figure 1C) close to the necrotic area were dark and resembled phenolic substance. In the (control) pH6.0 features of the leaf margin (Figure1D), revealed intact epidermal and mesophyll cells. SAR treated leaf margin at pH3.0 presented distorted epidermis further exposing the palisade cells to damage (Figure 1E). At pH2.0, margin collapsed (Figure 1F), revealing necrotic tissues. Cells that make up the midrib looked healthy at pH6.0 (Figure 1G); collapsed and necrotic tissue was observed at pH 3.0 (Figure 1H); ruptured and collapsed cells was found in necrotic areas at pH2.0 (Figure 1I).

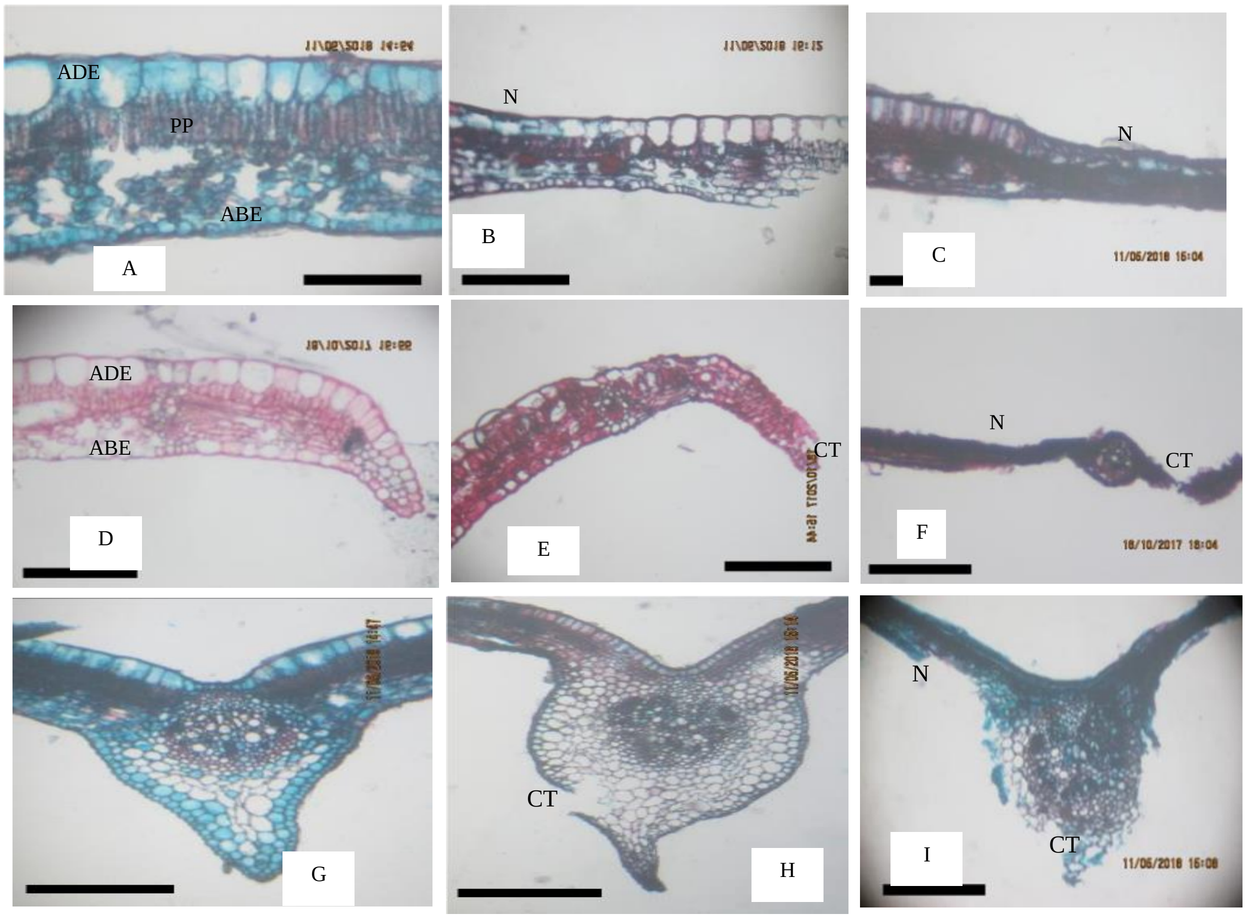

Figure 1**:** Leaf transverse section of *Dioscorea alata*, (A, D and G) control treatment, (B, E and H) acid rain pH3.0, (C, F and I) acid rain pH2.0. (A) Lamina mid-region (380µm), (B) Start of necrosis (280µm), (C) Marginal cells not easily distinguishable (D) Leaf margin (610µm) (E) Start of marginal necrosis (600µm) (F) Marginal necrosis (620µm), (G) Midrib with healthy tissue (bar= 220 µm), (H) Cell collapse in midrib and veins at pH3.0 (bar= 230 µm), (I) Necrosis and cell rupturing at pH2.0 (bar= 220 µm). ADE – Adaxial epidermis, ABE – Abaxial epidermis, PP – Palisade parenchyma, N – Necrotic areas, CT- collapsed tissue.

Discussion

Simulated acid rain (SAR) pH2.0 and pH3.0 caused lesions on both adaxial and abaxial surfaces of yam leaves investigated. Edosa and Bamidele, (2021) reported significant foliar damage, chlorophyll decline and growth retardation in *Dioscorea rotundata* in response to simulated acid rain at pH2.0. Acid rain precipitation used in this study caused necrosis and chlorosis in *D. alata* leaves at pH2.0, 3.0 and slightly at pH4.0 but was absent at pH6.0 (control). Rodriguez - Sanchez *et al*., (2020) reported that leaves of *Liquidambar styraciflua* displayed brown spots when sprayed with simulated acid rain of pH2.5. In this study, vine length, leaf number, leaf area and chlorophyll content index decreased significantly at pH2.0 relative to the control pH6.0. This is similar to the findings of Odiyi and Bamidele (2014) who reported that a cultivar of *Manihot esculenta* showed marked decrease in growth parameters when compared to the control. Harvest index of *D. alata* tubers decreased minimally at pH2.0 when compared to the control. 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. Percentage leaf surface area necrosis decreased with decrease in simulated acid rain concentration. Singh and Agrawal (2004) reported a reduction in leaf area of wheat (*Triticum aestivum*) Sonalika cultivar in response to simulated acid rain at pH3.0 and 4.0. Noticeable leaf injury was observed when *D. alata* was sprayed with acidic rain at (pH2.0 and 3.0). Sensitive plants to pollutants can present changes in their morphology, anatomy, physiology and biochemistry (Silva *et al.*, 2005b). The anatomical analysis of injuries caused by pollutants on plant species has been used in different studies to access the level of damage caused by pollutants (Carvalho-Andrade *et al*., 2020; Silva *et al*., 2005a). Acid solution deposited on leaves affect the epidermal cells causing erosion of cuticle and altering the leaf permeability (Du *et al*., 2017). In *D. alata* necrosis was located in the veins and in-between the veins as a result of rainwater accumulating in these regions. Rodriguez- Sanchez *et al*., (2020) reported that SAR caused necrotic spots in the intercoastal areas of *L. styraciflua* leaves at pH2.5. The cells near the necrotic area, showed changes in the palisade and spongy mesophyll. The structures of the epidermal and mesophyll cells within an injured area lesion were indistinguishable but healthy cells occurred immediately adjacent to collapsed necrotic region (Silva *et al*., 2000). Dark contents which might be phenolic substances were observed in damaged leaf tissue. 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.

Conclusion and recommendation

In this study, acid rain exposure caused morphological changes in *D. alata* including chlorosis, and necrosis. Simulated acid rain treatment at pH2.0 and pH3.0 had inhibitory effect on leaf (length, number, area and chlorophyll) of *Dioscorea alata* as compared with the control. The presence of cell damage using microscope revealed collapse in the adaxial and abaxial epidermis, mesophyll layer, resulting in a decrease in foliar growth. There is need for further studies on the effects of acid rain on growth, productivity, leaf stomata and photosynthesis of other economic crops. For agricultural land affected by acid rain, soil improvement measures like applying lime to the fields, selecting acid tolerant plants offer solution to achieving more production. Policy makers, Government, Environmental agencies need to put measures in place to curb emissions, thereby ensuring a safer environment for our crops to thrive in.

References

Abbasi, T., Poornima, P., and Kannadasan, T. (2013): Acid rain: Past, present and future. *Int. J. Environ*. Eng, 5: 229-272.

Agueguia, A. (1993): Non Destructive estimation of leaf area in cocoyam (*Xanthosoma sagitifolium (L).* Scohott). *Journal of Agronomy and Crop Science,* 171 (2): 138-141.

Ayanwuyi, E., Akinboye, A. O., and Oyetoro J. O. (2011): Yam production in Orire Local government Area of Oyo State, Nigeria: Farmers perceived constraints. *World Journal of Young Researchers,* 2:16-19

Banwart, W. L., Finke, R. L., Porter, P. M., and Hassett, J. I. (1990): Sensitivity of twenty soybean cultivars to simulated acid rain. *Journal of Environmental Quality,* 19: 339-346.

Carvalho-Andrade G., Nalon Castro L., and Campos da Silva L. (2020): Micromorphological alterations induced by simulated acid rain on leaf surface of *Joannesia princeps* Vell. *Ecological Indicators*, 116,106526

Cumpertz M. L., Tingey D. T., and Hogsett W. E. (1982): Precision and accuracy of visual foliar assessment. *Journal of Environmental Qual*ity, 11: 549-553.

Du, E., Dang, D., Xuetong, Z., Xiaofei, J., Wim, V. (2017): Direct effect of leaf acid rain on leaf chlorophyll contents of terrestrial plants in China. *Sci. Total Environ*, 15: 605-606.

Edosa, E. P., and Bamidele, J. F. (2021): Simulated acid rain impact on growth, yield and leaf anatomy of *Dioscorea rotundata* (L.). *Agricultural Science and Technology*, 13(2): 176-180.

Efe, S. I. (2005): Urban effects on precipitation amount, distribution and rainwater quality in Warri Metropolis. Ph.D. Thesis. Department of Geography and Regional Planning, DELSU – Abraka. Pp.10-103.

Efe, S. I. (2010): Environment and Sustainable Development: the case of Climate Change and Flood hazards in The Niger Delta Region of Nigeria, Proceedings, International Conference on Natural Resource, Security and Development in the Niger Delta, Niger Delta Wetlands Center, Yenagoa, Bayelsa State, Nigeria.

Evans, L. T. (1993): Crop evolution, adaptation and yield. Cambridge University Press, Cambridge University Press Cambridge.

Huang, X., Zeng, O., and Zhon. Q. (2005): Effect of acid rain on seed germination of rice, wheat and grape. *Huanjingkexne,* 26(1): 181-184

Hunt, R. (1990): Basic growth analysis: Plant growth analysis for Beginners. Academic Press, London, U. K. Iglesias, C. A., Calle, F., Hershey, C., Jaramillo, G., and Mesa E. (1994): Sensitivity of cassava (*Manihot esculenta* Crantz.) clones to environmental changes. *Field Crops Res*. 36: 213-220.

Kidd, J., and Kidd, R. (2006). Air pollution problems and solutions. Chelsea house publishers, New York, pp.74-83

Ma, Y., Wang, B., Zhang, R., Gao, Y., Zhang, X., Li, Y., Zuo, Z. (2019). Initial simulated acid rain impacts reactive oxygen species metabolism and photosynthetic abilities in *Cinnamonum camphora* undergoing high temperature. *Ind.Crop. Prod*, 135: 352-361.

Nwike M. C., Okoli T. O., Ugwumba, C. O. A. (2017): Economics of Water Yam (*Dioscorea alata*) Production in South Eastern Nigeria. *Journal of Agriculture and Veterinary Science*, 10 (7): 13-18.

Odiyi, B. O., and Bamidele, J. F. (2014): Effects of simulated acid rain on the Growth and yield of Cassava *(Manihot escullenta). Journal of Agricultural Science* 6: 9-10.

Rodriguez- Sanchez, V. M., Ulises, R., German, C., and Estela, S. (2020). Does acid rain alter the leaf anatomy and photosynthetic pigments in urban trees? *Plants,* 9, 868-878.

Silva, L. C., Azevedo, A. A., Silva, E. A.M., and Oliva, M. A. (2000): Flúor em chuvasimulada: sintomatologia e efeitos sobre a estrutura foliar e o crescimento de plantas arbóreas. *Revista Brasileira de Botânica*, 23: 383-391

Silva, L., Azevedo, A., Silva, E., and Oliva, M. (2005a): Effects of simulated acid rain on the growth and anatomy of five Brazilian tree species and anatomy of the most sensitive species (Joannesia princes). *Australian Journal of Botany*, 53: 789-796.

Silva, L., Oliva, M., Azevedo, A., Araújo, J., and Aguiar, R. (2005b): Micromorphological and anatomical alterations caused by simulated acid rain in Restinga plants: *Eugenia uniflora* and *Clusiahilariana*. *Water, Air and Soil Pollution*, 168: 129-143.

Singh, B., and Agrawal, M. (2004): Impact of simulated acid rain on growth and yield of two cultivars of wheat. *Water, Air and Soil Pollution,* 152: 71-80.

Weathers, K. C., and Likens, G.E. (2006): Acid rain In: Rom, W.N. (ed.) Environmental and occupational medicine. Lippincott – Raven publisher, Philadelphia. pp. 1549-1561.

Wireko – Manu F., Ibok O. William O., Robert A., and Bussie Maziya – D. (2013): Potential health benefits of water yam (*Dioscorea alata*). *Food Funct.* 4: 1496-1501.

Zobel, A., Nighswander, J. E. (1991): Accumulation of phenolic compounds in the necrotic areas of Austrian and Red Pine Needles after spraying with sulpuric acid: a possible bioindicator of air pollution. *New phytology*. 117: 565-574.