

**STUDY OF THE IMPACT OF ACIDULATED RAINWATER ON LEAF TISSUES OF  
TOMATO (*Solanum lycopersicum* Linn.) AND PEPPER (*Capsicum annum* Linn.)**

**BY**

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## Certification

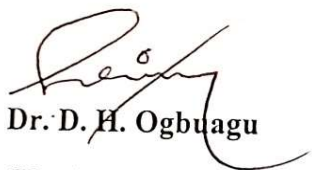
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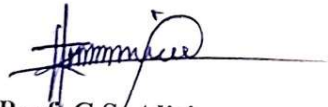
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## **Dedication**

I dedicate this work to God Almighty who in His mercy and loving kindness has seen me through and to my parents for their financial support.

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## Abstract

The acidulation of rainwater is connected intimately with industrial development. The leaves of Tomato and Pepper were examined for the impact of acidulated rainfall on their morphology, anatomy, and physiology. Rainwater samples collected from industrial and automobile traffic congested Cities of Lagos, Port Harcourt, Kano, and Gombe (control) were used in a screen-house experiment. The effect of atmospheric gases  $\text{NO}_2$ ,  $\text{SO}_2$ , and  $\text{CO}_2$  was determined on morphology, anatomy of phylloplane, photosynthetic chlorophylls, and agro-morphology of the plants. Statistical package R version 4.2.1 was used to analyze data. Duncan's multiple-range test was used to determine the significance of the mean difference. The findings showed that from April through July, the concentrations of atmospheric acid derivatives were significantly ( $p < 0.05$ ) decreased. Morphological changes such as physical damage to the leaf, formation of white Scars, and necrosis were observed. Anatomical changes such as alterations on the cuticle and collapsed epidermal cells and the formation of lobules of scarred tissue were observed. Plants heights were significantly reduced at ( $p < 0.05$ ) ( $F_{\text{Tomato}}=7.8894$ ;  $F_{\text{Pepper}}=63.835$ ), leaf area was also significantly decreased ( $F_{\text{Tomato}}=16.341$ ;  $F_{\text{Pepper}}=60.965$ ;  $p < 0.05$ ), stem girth were also significantly ( $p < 0.05$ ) reduced ( $F_{\text{Tomato}}=8.8174$ ;  $F_{\text{Pepper}}=39.3$ ), number of leaf ( $F_{\text{Tomato}}=22.482$ ;  $F_{\text{Pepper}}=34.265$ ), Relative growth rate ( $F_{\text{Tomato}}=38.522$ ;  $F_{\text{Pepper}}=40.646$ ) and chlorophyll content ( $F_{\text{Tomato}}=8.4128, 2.5368, 11.411$ ;  $F_{\text{Pepper}}=4.6029, 7.8154, 36.746$ ) were also significantly ( $p < 0.05$ ) decreased. The findings showed that both car emissions and heavy industrial activities contributed significantly to the acidity in rainwater. It also demonstrated that acidified rainwater significantly affects the morphology, anatomy, and physiology of plants studied. It is, therefore, important to plant acidophilic trees in the study areas to reduce the effect of acid rain on other vegetable crops.

**Key words:** Acid rain, Anatomy, Physiology and Morphology.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background to the study

Environmental implications of industrialization include acid rain and air pollution (Burns, Aherne, Gay, & Lehmann, 2016). Several inventions that make Man's life easier also lead to pollution, (Grennfelt, Engleryd, Forsius, Hov, Rodhe, & Cowling, 2020), by releasing harmful substances such as sulfur dioxide, Nitrogen dioxide, and carbon dioxide into the environment (Chandra, Mainpuri, & Pradesh, 2017).

Energy is the vital hub for numerous sets of man's activities and is necessary for the generation of end products from natural resources (Tiwari, & Ghosal, 2017). Unfortunately, many energy sources also produce the majority of harmful environmental products, such as pollution, global warming, acid rain, and radioactive wastes (Gilani, 2018).

Acid rain is an atmospheric precipitation that is abnormally acidic and contains high-level  $H^+$  ions (Lal, 2016). This is formed by emissions of Sulfur dioxide ( $SO_2$ ) and Nitrogen (IV) oxide ( $NO_2$ ) (IEA, 2016). These atmospheric pollutants dissolve in atmospheric vapour to produce acid rain (Chandra *et al.*, 2017). Thereby reducing the pH level below 5.6 (Dondapati, Reddy, & Recharla, 2013). Urban areas and heavily industrialized areas have experienced problems with acid rain. (Grennfelt *et al.*, 2020). Globally, burning fossil fuels to produce power, heating, transportation, and industry generates most air pollutants (US EPA, 2017). Although rain is naturally acidic (Chandra *et al.*, 2017), it is being increasingly acidified by anthropogenic activities such as pollution from homes, factories, power stations, and automobile vehicles (Grennfelt *et al.*, 2020), which results in the release of substances such as Carbon dioxide ( $CO_2$ ), Nitric dioxide ( $NO_2$ ) and Sulfur dioxide ( $SO_2$ ) (Chandra *et al.*, 2017).

Carbon dioxide is present excessively in the atmosphere (Chandra *et al.*, 2017) and, therefore contributes more to the natural acidity of rainwater (Balasubramanian, Udayasoorian, & Prabu,

2007). Carbon dioxide is hydrolyzed to become carbonic acid and then deionized to form hydrogen ions ( $H^+$ ) and hydrogen carbonate ions ( $HCO_3^-$ ). The formation of  $H^+$  gives the solution characteristic of acid rain (Chandra *et al.*, 2017), thus lowering the pH of a solution (Kim, Bae, Cho, & Kang, 2014).

Nitric oxide (NO), is also involved in the acidification of rainwater (Chandra *et al.*, 2017), it is formed by the reaction between oxygen and nitrogen during a lightning storm in the atmosphere (Veronica, *et al.*, 2020). Nitric oxide is then oxidized to form nitrogen dioxide ( $NO_2$ ), thus hydrolyzed to form nitric acid (IEA, 2016). This dissociates in water to form hydrogen ions ( $H^+$ ) and nitrate ions ( $NO_3^-$ ) thus, reducing the pH level of the solution (Chandra *et al.*, 2017).

Although sulfuric oxide is formed in little quantity through the biological decay of organic matter and volcanic activities (Veronica, Rosas, & Estela, 2020), it is produced almost entirely by human activities (Chandra *et al.*, 2017), particularly from the combustion of fossil fuels containing sulfur in power plants (IEA, 2016). After burning these fossils, the sulfur contained in them oxidized to form sulfur dioxide (US EPA, 2017). Sulfuric acid is a strong acid (Mohajan, 2014), so it is readily ionized in water, to produce  $H^+$  ion and  $HSO_4^-$  ion (Chandra *et al.*, 2017). The  $HSO_4^-$  ion continuously dissociates to form  $H^+$  and  $SO_4^{2-}$ . Thus, the presence of sulfuric acid increases the level of  $H^+$  dramatically, and so causes a drop in the pH level of the solution (Chandra *et al.*, 2017).

Acid rain is considered a global threat (IEA, 2016), affecting forests, lakes, and farmlands (Ma, Wang, Zhang, Gao, Zhang, Li, & Zuo, 2019). It is reported that industrial activities are the major source of atmospheric pollutants (Burns *et al.*, 2016), and other contaminants are created by the influence of light, temperature, or humidity (Abbasi, Vasileva, & Lu, 2017). The accumulation of strong acids and other oxides in the atmosphere, which primarily comes from human activity in the form of rain, snow, gas, or particles, is one example of how industrial activities have an impact on the environment (Li, Yang, Ahammed, Shen, Yan, Li, & Han, 2016). These substances arise mainly from the combustion of fossil fuels with a higher level of sulfur content (IEA, 2016), and the processing of metallic minerals (Eguagie, Aiwansoba, Omofomwan, & Oyanoghafo, 2016).

Acid rain causes damage to the leaves, stems, and roots of a plant (Huang, Wang, Zhong, Huang, Fu, Wang, & Teng, 2019), reduces crop yields (Awasthi, Saha, Regon, Sahoo, Chowra, Pradhan, Roy, & Panda, 2017), decreases the photosynthesis by plants (Debnath, Li, Liu, Pan, Ma, & Qiu, 2020), and reduces their germination and chlorophyll Content (Sun, Hu, Li, Wang, Zhou, & Huang, 2016). Acid rain is reported to penetrate through the cuticle and cause internal damage to leaf tissue (Liu, Fu, Zhang, Zhai, Meng, Lin, Zhuang, Wang, & Zhang, 2018). Monocotyledons survive less impact of the effect of acid rain than dicotyledons (Lal, 2016). However, it has been noted that immature plants' rootlets, leaves, and shoots are usually more sensitive to low-pH environments (Lal, 2016). It has been suggested that acid rain slows plant growth by causing anomalies in physiological processes including photosynthesis, chlorophyll content (Sun, Hu, Li, Wang, Zhou, & Huang, 2016), nitrogen metabolism (Ramlall, Varghese, Ramdhani, Pammenter, Bhatt, & Berjak, 2015), and production of reactive oxygen species (Martins, Gonçalves, & Romano, 2013). There are, however, some rare instances of growth promotion (Zhang, Yi, Gao, Wang, Shao, Liv, Chena, Liu, & Shen, 2020). The reduction of chlorophyll content of a leaf is an indicator of direct foliage damage (Sun *et al.*, 2016). Thus, it is strongly related to plant growth and enables them to photosynthesize (Ramlall *et al.*, 2015), which is substantially reduced by low pH (Liu *et al.*, 2018). Specifically, most of the plant agro-morphological parameters are influenced by pH (Jiang, Li, Zeng, Wei, & Yu, 2017).

Anatomically, acid rain is reported to alter the cuticle thickness (Eichert, & Fernández, 2012), deformation of cell and stomatal cells occlusion (Ramlall *et al.*, 2015), removal of trichomes, cellular collapse, and formation of scar tissues (Lebedev, Krutovsky, & Shestibratov, 2019). Alteration of the cuticle generates a hydrophilic condition (Yang *et al.*, 2015), thus, affecting the permeability of the leaf, making it more sensitive to water loss, or permitting the entry of a more acidic solution (Eichert *et al.*, 2012). Acid rain has a vast effect on photosynthesis and growth (Yang *et al.*, 2015), it makes plants more susceptible to disease and drought, and a process known as "dieback" causes leaves to turn brown and drop off (Xu, Pan, Yao, Feng, Wu, & Shao, 2020). It also causes damage to the root system (Lal, 2016), affects root mycorrhiza, and decreases the lichens (Rahman, Lee, Ji, Kabir,

Jones, & Lee, 2018). Phosphorus is also reduced as a result of acid rain (Robles-Aguilar, Ana, Pang, Postma, Schrey, Lambers, & Jablonowski, 2019), which also inhibit fruit production and the accumulation of toxic heavy metal such as zinc and aluminum (Rahman *et al.*, 2018), thus, affecting the plant root growth and reduction of chlorophyll (Awasthi *et al.*, 2017).

Carotenoids are the most widespread naturally occurring yellow, orange, and red pigments (Martini, D'Addario, Bonechi, Leone, Tognazzi, Consumi, Magnani, & Rossi, 2010). Carotenoids are pigments found exclusively in plants, algae, fungi, and bacteria (Yabuzaki, 2017). In plants, carotenoid is responsible for photosynthesis as well as the characteristic color of fruits and flowers (Kalac, 2012). Carotenoids are related to various health benefits, mainly as an antioxidant (Ucci, Tomo, Tritschler, Cordone, Lanuti, Bologna, Di Silvestre, Di Pietro, Pipino, & Mandatori, 2019), anti-inflammatory (Kawata, Murakami, Suzuki, & Fujisawa, 2018), and immunomodulatory compounds (Toti, Chen, Palmery, Villaño Valencia, & Peluso, 2018). Carotenoids are sensitive to environmental conditions such as light, temperature, oxygen as well as acidic conditions (Shi, Xue, & Wang, 2015).

According to reports, crops are more vulnerable than natural plants (weeds) when acid rain directly destroys their leaves (Huang *et al.*, 2019). For example, tomato plant plays a significant part in agriculture since it is used by a wide range of people as a source of vitamins, vegetables, fresh fruit, cosmetics, and medications (Raiola, Rigano, Calafiore, Frusciante, & Barone, 2014).

## **1.2 Statement of the problem**

The cause of poor water quality is connected to natural processes, but it is more frequently tied to human activity and intimately associated with industrial development (Burns *et al.*, 2016). There are particular water quality standards for drinking water, agriculture, and industry. Species diversity declines as a result of water quality degradation (Debnath *et al.*, 2020). Changes in water quality metrics like nutrient concentrations, temperature, dissolved oxygen levels, and pH are linked to these changes in environmental quality. Acid rain is reported to penetrate the leaf through the cuticle, and

cause internal injuries, damaging the leaves, stems, and roots of plants, reducing crop yields, decreasing photosynthesis in plants, and reduces their germination and chlorophyll Content (Sun *et al.*, 2016). However, there is a need to study the elements of resilience in plants (Tomato and Pepper) due to the rise of cities and the ongoing need to improve the environmental quality of its inhabitants. The purpose of this study is to examine the chemical composition of rainwater collected from an area that has been contaminated by industrial activity, as well as the traffic-congested areas of production and transportation. However, it sought to identify potential fruit species to cultivate in industrialized and traffic-congested locations.

### **1.3 Justification of the Study**

This study examines the morphological, anatomical, and physiological resilience in Tomato and Pepper due to the rise of cities and the ongoing need to improve the environmental quality of its inhabitants. However, the research adds to sustainable development in regions throughout the world that is affected by acid rain and has both scientific and practical ramifications.

### **1.4 Aim and Objectives**

This study aimed to ascertain the effects of acid rain on *Solanum lycopersicum* and *Capsicum annum* leaf tissues. The Specific objectives are to;

1. Determine the pH level and concentrations of acid derivatives (NO<sub>2</sub>, SO<sub>2</sub>, and CO<sub>2</sub>) in rainwater harvested from selected industrialized Cities in Nigeria.
2. Assess the morphological and anatomical properties of tomato and pepper irrigated with acidulated rainwater.
3. Determine the photosynthetic chlorophyll (a and b) contents of the tomato and pepper irrigated with acidulated rainwater.
4. Evaluate of the impact of acidulated rainwater on the agro-morphology of the plants.

### **1.5 Research Questions**

The following research questions would be utilized in this study:

- Does the amount of acid derivatives in the atmosphere increase as a result of emissions from industries and areas with heavy traffic?
- Does rainwater from busy roads and industrial areas have an impact on leaf tissues?
- Does rainwater from busy roads and industrial areas have an impact on photosynthesis of the plants?
- Does rainwater from busy roads and industrial areas have an impact on plant growth?

## **1.6 Research Hypothesis**

H<sub>01</sub>: Acid derivatives in ambient air are increased by emissions from industrial areas.

H<sub>02</sub>: Rainwater from locations with heavy industrial and automotive traffic does not significantly affect (change) leaf tissues.

H<sub>03</sub>: Chlorophyll content is not directly impacted by rainwater from locations with heavy industry and automotive activity.

H<sub>04</sub>: The development of the two test plant species is affected by rainwater from locations with heavy industrial and vehicular traffic.

## **1.7 Scope of the study**

This study was conducted in a screen house at the Federal University of Technology Owerri. Imo State, southeastern part of Nigeria. The study covers the determination of pH levels and concentrations of acid derivatives (SO<sub>2</sub>, NO<sub>2</sub>, and CO<sub>2</sub>) of atmospheric gases in rainwater harvested from industrialized Cities of Lagos, Port Harcourt, Kano, and Gombe (Control). However, the study examines the morphological and anatomical properties of plants irrigated with acidulated rainwater collected from those Cities. Again, the chlorophyll contents of the plants irrigated with acidulated rainwater were studied. Moreover, the impact of acidulated rainwater on the agro-morphology of plants was studied.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Acid rain

Precipitation that contains high levels of hydrogen ions is known as acid rain (Lal, 2016), this is created by emissions of sulfur dioxide and nitrogen oxide from burning fossil fuels, smoke from automobiles, and emissions from industrial processes, which solubilizes in the atmosphere to produce acid rain (Chandra *et al.*, 2017). However, Robert Angus Smith (1872) named the low pH solution “acid rain” when he discovered that it causes damage to leaves (Lal, 2016). When acid is solubilized, the pH level drops below 5.6 (Chandra *et al.*, 2017). Acid rain can affect terrestrial environments in several other ways, including acidification of soil and the alteration of nutrient supply (Lal, 2016).

Mining alters the air, soil, vegetation, and organisms that cover excavation sites, which causes harm to natural ecosystems (Gasiorek, Kowalskam, Mazurek, & Pająkm, 2017). As a result, mining presents toxicological difficulties for nearby ecosystems. Mining has the potential to harm the environment, including acidification (Abiya, Odiyi, Ologundudu, Akinnifesi, & Akadiri, 2019). Acid rain is reported to contain gaseous oxides such as sulfur dioxide, nitrogen oxides, carbon dioxide, and chloride either in form of particle or gas (Burns *et al.*, 2016). These waste products react with the environment to produce sulfuric acid, nitric acid, carbonic acid, and hydrochloric acid (Chandra *et al.*, 2017). pH is a measure of acidity on a logarithmic scale. A pH of 5 rainwater is ten times more acidic than a pH of 6. Clean rain has a pH of 5.6, which is a moderate acidity, while seawater has a pH of 8.3, which is mildly alkaline (UK Environmental Protection and Water Statistics, 2019).

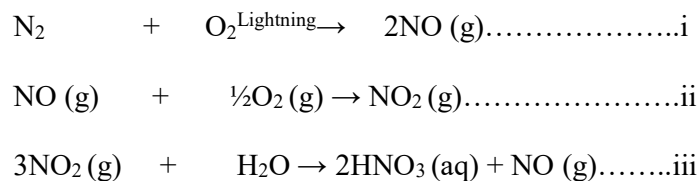
Acid rain is reported among the greatest threats to the plants and environment at large. The contamination by sulfuric acid and nitric acid causes the concentration of hydrogen ions to rise. As a result, aquatic habitats' pH (potential hydrogen) scales are reduced (Zhang *et al.*, 2017). However, it inhibits plant growth and yield by posing abnormalities in the metabolism of plants. Again, it reduces

soil nutrients including N, Ca, Mg, and K. Acid rain enhances the soil's toxicity of heavy metals like Al, Hg, Mn, Cd, and Pb, which harms or kills plants and trees.

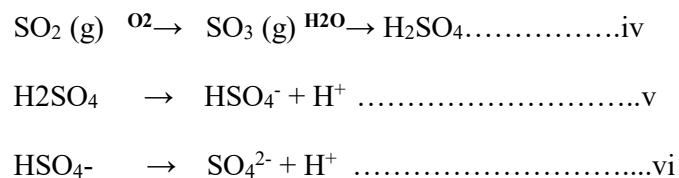
## 2.2 Causes of Acid Rain

### 2.2.1 Industrialization/Urbanization

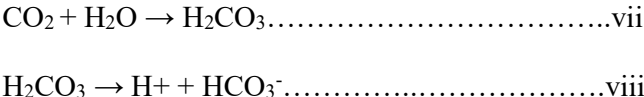
Most of the sources of acidic rain are the result of emissions from industrial activities, vehicles, and pollution formed from man's anthropogenic activities (Burns *et al.*, 2016). However, these produced more air pollutants than naturally occurring sources of acidity, including sulfur, nitrogen, and carbon oxides (Chandra *et al.*, 2017). Nitric oxide, which is one of the acidity-forming oxides, is produced by the oxidizing reaction of nitrogen gas during a lightning storm in the atmosphere (Kumar, 2018). As a result, nitric acid (HNO<sub>3</sub>) is created when the nitrogen dioxide generated combines with water. However, the pH of the solution was lowered as a result of this acid's deionization in water, which produced hydrogen and nitrate ions.



Even though sulfuric acid is mostly created by burning fossil fuels to produce electricity and sporadically by natural processes like the biological decay of organic matter and volcanic explosions (Kumar, 2017), sulfur gas oxidizes in the presence of oxygen to form sulfur dioxide. Thus, it hydrolyzed to form sulfuric acid, hence de-ionized to produce hydrogen and sulfate ion. This hydrogen ion concentration may have an impact on biological membranes, electron transport mechanisms, and pH-specific biochemical reactions (Lal, 2016).



Carbon dioxide is produced when carbon undergoes an oxidizing reaction with oxygen to make carbonic acid (Burns *et. al.*, 2016). When carbon dioxide reacts with water, it creates carbonic acid, which de-ionizes to produce the ions hydrogen and carbonic ion. Hydrogen ion decreases the pH of the solution hence making it an acidic solution. It has been seen those abnormalities in the metabolisms of plants (photosynthesis, transpiration, etc.) can result from exposure to these acidic conditions (Lal, 2016).



Nigeria is one of such countries with a high ranking for acid-rain ecological sensitivity. Rapid industrialization has taken place in this sub-Saharan African country within the last few decades, leading to increased loads of pollutant emissions. Industrial waste management, though legislated, is not effectively enforced, such that much of what is generated as waste is discharged untreated into the environment.

**2.2.2 Combustion of fossil fuel (Non-renewable energy)**

The harnessing of energy from fossil fuels produces the majority of greenhouse gases as well as rapidly poses climate change (Wuebbles, Fahey, Hibbard, DeAngelo, Doherty, Hayhoe, Horton, Kossin, Taylor, & Weaver, 2017). Most countries of the world depend solely on fossil fuels as an alternative source of energy (Sugiawan, & Managi, 2019), which yielded many undesirable effects on the environment including global warming as well as air pollution (Lott, Pye, & Dodds, 2017). However, pollution of air poses negative impacts on both humans and vegetation (US EPA, 2017). About 35 billion tons of carbon dioxide is generated as a result of human activities globally (IEA, 2016). However, it will be in higher concentration in the atmosphere many years later. Although the emission of carbon dioxide from natural gas is significantly less toxic compared to that of fossil fuel (NETL, 2015), spillage of crude oil through transportation, extraction as well as processing contributes to the release of methane in the environment which is 34 times toxic than carbon dioxide

(Myhre, Shindell, Bréon, Collins, Fuglestedt, Huang, Koch, Lamarque, Lee, & Mendoza, 2013). The use of fossil fuel as an alternative source of energy surpasses the use of natural gas and other eco-friendly sources such as solar energy (IEA, 2016).

### **2.3 Impact of acidulated rain water on soil properties**

Increase of  $H^+$  in soil by acid rain leads to the displacement of the base cat-ions in the soil and the depletion of these cat-ions may be irreversible (Xie, Du, Zeng, Li, Yan, & Jiao, 2009). However, acid rain's sulfuric and nitrate ions accelerate the leaching of base cat-ions from the soil (Zhang, Ouyang, & Ling, 2007). This depletion of base cat-ions decreases the nutrient content in the soil and hence reduces soil fertility (Liu, Fu, Zhang, Zhai, Meng, Lin, Zhuang, Wang, & Zhang, 2018). Additionally, it postpones the soil's and surface water's nutritional recovery after acidification. (Zhang *et al.*, 2007). In absence of remunerations for depleted base cat-ions, the acidification of soil might be posed. This leads to the occurrence of toxic persistent metals such as Al, and Fe among others; these metals inhibit the growth of plants in the soil (Zhang *et al.*, 2007; Qingyan, Jianping, Guohua, Juxiu, Guowei, Guoyi, & Deqiang, 2015). In a monsoon evergreen broad-leaved forest in southern China, researchers studied the impact of simulated acid rain on soil and soil solution chemistry. After applying simulated acid rain, a laboratory experiment was conducted to evaluate the impact of acid rain on soil solution and soil chemistry (SAR). Five different pH levels (2.5, 3.0, 3.5, 4.0 and 4.5 as control) were used. The result revealed that the acidification of soil occurred at pH 3.5. However, the concentration of  $NO_3^-$ ,  $SO_4^{2-}$ , and  $Ca^{2+}$  significantly increased at pH 3.5. Exchangeable  $Al^{3+}$  level rise with a rise in acidity of the soil. pH 2.5 induced soluble Al and Fe, 19.6 and 5.5 times respectively to the soil. They concluded soil nutrients are sensitive to the acid rain. However, the impacts of acid rain on soil solution chemistry depend on intensity as well as the duration of simulated acid rain. pH 4.5 has less effect on soil and soil solution in short term, while the leaching of soil and soil chemistry occurred in precipitation between pH 3.0 and 3.5.

## **2.4 Impacts of acid rain on plant**

### **2.4.1 Effects on leaves' internal tissues.**

According to reports, acid rain penetrates the cuticle of leaves and causes internal and external morphological deformities. It can also harm plants' leaves, stems, and roots, as well as decrease photosynthesis by lowering the chlorophyll content of leaves (Debnath *et al.*, 2020). Anatomically, low pH poses defects of internal tissues such as occlusion of stomata, cellular deformation, and formation of scar tissues (Lebedev *et al.*, 2019). Alteration of the cuticle generates increase in the loss of water by plants (Yang *et al.*, 2011). The structural changes in leaves, however, also affect the size of the adaxial epidermis, the length of the adaxial epidermis, the width of the abaxial epidermis, the length of the abaxial epidermis, the thickness of the mesophyll, the thickness of the phloem, the thickness of the xylem, the diameter of the vessel elements, the size of the form cells, the size of the form (Tulay, Adnan, & Cengiz, 2018).

Low pH, however, reduces the growth of the plant and alters the function of the plasma membrane by increasing  $Al^{3+}$  to a toxic concentration. It also results in the loss of essential nutrients such as Ca, Mg, and P in the soil (Ma, 2017).

### **2.4.2 Effects on plant photosynthesis**

One of the abiotic stressors that harm plants is acid rain. Thus, exposed plants suffered from foliar injuries, and anatomical modification (Li, Manning, & Wang, 2019), by causing modifications to the structure of the photosynthetic pigment apparatus and a decrease in chlorophyll concentrations, thereby reducing the processes of photosynthesis and chlorophyll synthesis (Sant' Anna-Santos, Silva, Azevedo, & Deque-Brasil, 2006). Reactive oxygen species produced by acid rain have an impact on plant physiology as well because they cause oxidative stress in the plants (Syed, Kapoor, & Bhat, 2018). High levels of reactive oxygen species can result in the oxidation of protein, inhibition of enzymatic activities, nucleic acid impairment, and cell death (Adrees, Ali, Rizwan, Ibrahim, Abbas, Farid, Zia-ur-Rehman, Irshad, & Bharwana, 2015). Although leaf area increases the

rate of photosynthesis, it also increases the plant's dry mass (Li *et al.*, 2019). Environmental factors such as temperature and acid rain have an impact on leaf area (Masarirambi, Mbokazi, Wahome, & Oseni, 2012); Rehman *et al.*, (2017) stated that acid rain stress inhibits the uptake and metabolism of nitrogen. Stomatal opening, chlorophyll synthesis, and chloroplast development were also negatively affected when exposed to acid rain (Gomez- sagasti, Marino, Khan, & Anjum2015).

The study by Zhang *et al.* (2014) examined how *Citrus sinensis* and *Citrus grandis*' physiological parameters related to photosynthesis and other processes were affected by low pH. Different pH levels of acidic rain were used to irrigate the plants (2 to 6). According to their research, the test plant has adapted to low pH. At pH 2.5, plant growth was limited. At pH 2.5, it was also possible to see bleach mottling and a decrease in chlorophyll on the leaves. So, compared *C. grandis*, with *C. sinensis* were marginally more tolerant to low pH.

The impact of simulated acid rain (SAR) on tomato growth, yield, and physiological parameters was studied by Shaukat *et al.* in 2018. The stimulated acid rain (pH 3.0 & 4.0) decreased chlorophyll content, and the length of the shoot and plants' dry weight were also reduced. They concluded that the impact is more profound at low pH (3.0)

Kausar, Hussain, & Khan, (2019) observed in their research that stimulated acid (pH 3.0 through 6.0) produced morphological defects, inhibition in biochemical processes, and alteration of epidermal tissues. At low pH, growth indices such as shoot length, leaf count, stem girth, and dry weight were inhibited. However, profound inhibition was observed at pH 3.0. Additionally, they noticed that the photosynthetic pigments (Chlorophyll a and b, total Chlorophyll, and carotenoids), seed protein, soluble and insoluble carbohydrates, and leaf epidermal characteristics (stomata quantity, position, and length) were all hindered by low pH.

### 2.4.3 Effects on plant growth

Because of one crucial element, the plant's water transport system, acid rain may significantly impact on plant's ability to grow (Yang *et al.*, 2015). Transpiration, photosynthesis, and the dispersion of organic or inorganic molecules throughout the plant's xylem, the vascular tissue that conducts water upward from the root, are just a few examples of the many processes that practically all plants go through to develop (Kim, Bae, Cho, & Kang, 2014). Low pH negatively affects this system, the plant will not be able to grow or function properly (Awasthi *et al.*, 2017).

Plant growth was directly hampered by low pH levels due to increased H<sup>+</sup> activity (Bian, Zhou, Sun, & Li, 2013). By encouraging the buildup of too many reactive oxygen species (ROS), such as superoxide radicals (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in plant tissues, a high H<sup>+</sup> concentration causes typical oxidative stress on plants. (Liu *et al.*, 2011). Complex antioxidant systems, including antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DR), as well as antioxidants like α-tocopherol, ascorbate, and reduced glutathione, have evolved in plants to prevent oxidative damage. According to studies, higher antioxidant enzyme activity levels may improve H<sup>+</sup> tolerance by enhancing the capacity to defend against oxidative damage (Chen, Liu, Wu, & Zheng, 2013).

The effects of simulated acid rain (SAR; sulfuric acid-based solutions with 4.5, 3.5, and 2.5 pH) on the yield and yield components of tomato (*Lycopersicon esculentum*) under greenhouse conditions were studied by Dursun, Kumlay, Yildirim, & Guvvenc, (2014) as well. Throughout the 75-day tests, the simulated acid rain was sprayed 27 times. The outcome demonstrates that pH 2.5 and 3.5 cause necrotic lesions and wilting of leaves, respectively. As a result, pH readings of 4.5 and 5.9 revealed no physical harm to plants. However, compared to the control, yield indices like fruit number, average fruit weight, fruit diameter, and fruit weight decreased at pH 2.5. In low pH, the shoot's length and diameter also decreased.

Low pH-related effects on yield, root growth, and nutrient uptake in black pepper (*Piper nigrum L.*) were studied by Chao *et al.*, (2014). The experiment was conducted in a greenhouse, the test plants were sprayed with pH (7.0, 5.5, 4.0, and 3.5). The results show that the maximum growth of the plant was at pH 5.5, while minimum growth was observed at pH 3.5. They concluded that the decrease in growth was a result of leached nutrients such as Ca and Mg due to acid rain.

According to Lee *et al.*, (2014). Study titled "potted plants grown in field chambers were used to compare responses of main crops to sulfuric acid rain." The plants were exposed to control rain and simulated sulfuric acid rain (pH 3.0, 3.5, or 4.0). According to the findings, out of 35 cultivars tested, leaves 31 suffered damage at pH 3.0, 28 at pH 3.5, and 5 at pH 4.0.

In a greenhouse study, Jacobson *et al.* (2015) treated radish plants with repeated doses of simulated acid rain with pH ranges between 2.6 and 5.0. In this investigation, the treatment consisted of using acidic solutions with sulfate-to-nitrate mass ratios of 2:1 and low pH values typical of the rainfall in the eastern United States. It has been discovered that rainfall exposures below pH 3.4 can induce foliar damage.

Ecophysiological Response of *Capsicum annuum L.* Exposed to Simulated Acid Rain was a study done by Bamidele *et al.* in 2015. A screen house experiment was conducted to evaluate the effects of low pH exposures on plants (2.0 through 5.0 and 5.6 as a control). Except for the control, the result demonstrates a negative impact on the plant's growth metrics (plant height, number of leaves, fresh and dried leaf weight, root, stem, and fruit weight). Fruit ripening and leaf area decreased. However, morphological flaws such as chlorosis, necrosis, early senescence, leaf abscission, and mortality were noted. The majority of these flaws were noticed as the pH level dropped.

Pragati, & Dhaka, (2016) conducted research on the effects of simulated acid rain on *Zinnia elegans* plants. Sulfuric acid and nitric acid were combined in a 3:1 ratio to create the simulated acid rain, which had pH values of 5.6, 4.5, 3.5, and 2.5. As pH levels drop, a decrease in plant height, root length, and dry weight are visible.

The impact of acid rain on the germination and growth of seedlings of *Vigna unguiculata ssp. sesquipedatis* (L.) Verdc was studied by Han (2019). The pH at which the acid rain was prepared varied (1.0, 2.0, 3.0, 4.0, 5.0, and 6.5). The outcome demonstrates that with a drop in pH, seed germination rate, germinated vigor, germination index, seedling root length, and hypocotyl length all tended to decline. When pH was 1.0, all *V. unguiculata ssp. Sesquipedatis* (L.) seeds had a musty smell.

The impact of simulated acid rain on soybean growth and yield was studied by Pham, Do, Tran, Bui, & Le, (2021). The plants were subjected to three times each week of simulated acid rain (pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0). They saw a decrease in the growth indicators like shoot length and branch count. However, the outcome demonstrates a drop in photosynthetic pigment concentration as pH falls. This is more obvious in plants exposed to acid rain with a pH of 3.0.

## **2.5 Choice of Exposed Plants**

Fruits are one of the six food classes, but they are a significant part of human nutrition as well as mythology, religion, and the arts. Fruits are not only sweet but also provide vital minerals for human diets (FAO, 2017). Recent dietary recommendations encourage increasing the consumption of fruit that contains phytochemicals. Fruits have positive impacts on human health and are crucial in preventing chronic illnesses (Zhang, 2015).

### **2.5.1 Nomenclature of Tomato (*Solanum lycopersicum* Linn.)**

- **Domain:** Eukaryota
- **Kingdom:** Plantae
- **Division:** Spermatophyta
- **Subphylum:** Angiospermae
- **Class:** Dicotyledonae
- **Order:** Solanales
- **Family:** Solanaceae

- **Genus:** Solanum
- **Species:** *Solanum lycopersicum*

(Sources: Beckles, 2012).

### **2.5.2 Growth stages of Tomato (*Solanum lycopersicum*)**

The growth of tomatoes is described in five stages by Jones (2013) and García, Van der Valk, & Elings, (2011). The germination and early growth as well as the development of initial leaves take place between 25 and 35 days; the vegetative period is between 20 to 25 days; the development of flowers occurs within 20 to 30 days; development of early fruit begins 20 to 30 days and mature fruiting 15 to 20 days. The period between each stage depends on the environmental condition such as air temperature, water, salinity/alkalinity, soil conditions, and availability of nutrients. Moreover, due to commercial need; these stages can be modified for specific climatic conditions which reduce the duration of growth of the plant. The average duration for the tomato to attain maturity depending on the variety is between 65 to 100 days; the estimated time for marketable maturity (early maturity) is between 50 and 65 days; while for late maturity is between 85 and 95 days (Jones, 2013).

### **2.5.3 Ecological Requirements of *Solanum lycopersicum***

#### **2.5.3.1 Temperature requirements**

Tomato attains high yield and quality in a relatively cool and dry climate. Thus, it is also adapted to varying temperatures (temperate, hot, and humid tropical). The optimum temperature for maximum yield for most cultivars lies between 21°C and 24°C. Extreme low and high temperatures (10°C and 38°C respectively) damage the tissue of the plant (Kittas, Karamanis, & Katsoulas, 2015). At extreme temperatures, the production of red pigment, lycopene, is inhibited (Naika, Van Lidt de Jeude, de Goffau, Hilmi, & Van Dam, 2015). Tomato requires periodic temperature (temperature difference between day and night) lies between 5°C to 7°C for at least 500 to 550 hours daylight (periods from germination to maturity) is an average of 6 hours daily for proper physiological

activities (Baudoin, Nono-Womdim, Lutaladio, Hodder, Castilla, Leonardi, De Pascale, Qaryouti, & Duffy, 2013).

### **2.5.3.2 Water and humidity requirements**

The tomato needs a sufficient supply of water for growth and development. The loss of leaves, flowers, and fruit occurs as a result of drought. Extreme humidity and rain, however, promote the formation of mold and fruit rot. Cloudy clouds prevent fruit from ripening (Shankara, Jeude, Goffau, Hilmi, & Dam, 2015).

### **2.5.3.3 Soil requirements**

Tomato requires nourished soil with good aeration and holding water capacity. It also requires well-drained, deep (15 to 20cm depth) sandy loam soil for proper growth and development. Tomato adopts to grow in moderately acidic conditions, but the optimum pH required for growth and development is reported between pH of 5.5 – 6.8 with adequate nutrient supply and availability. Moderate addition of organic matter also promotes the growth of tomatoes. Soil containing high organic matter such as peat soil is not suitable for the growth of tomatoes due to its high water-holding capacity (Shakara *et al.*, 2015).

### **2.5.3.4 Economic importance of *Solanum lycopersicum***

*Solanum lycopersicum L.* (Tomato), one of the most consumed plants in the world today, is also referred to as nutraceutical due to its antioxidant properties (Ray, Saha, Raychaudhuri, Chakraborty, 2016). It is a significant source of chemical substances, particularly secondary metabolites that have antioxidant properties, including, but not limited to, polyphenols, hydroxycinnamic acids, carotenoids, and vitamins. These substances are well-known for their use in the treatment of human illnesses like cardiovascular and neurological diseases (Chen, 2015) as well as some types of cancer (FAO, 2017). Tomatoes are said to grow in a variety of climates; however, they are typically

cultivated as annuals in commercial settings even though they can be produced as perennials or semi-perennials in open fields or greenhouses. (See Fig. 2.1)



**Fig 2.1: Tomato leaves**

(Source: OECD, 2017)

## **2.5.2 Sweet pepper (*Capsicum annum*)**

### **2.5.2.1 Nomenclature of *Capsicum annum* (Linn.)**

- **Domain:** Eukaryota
- **Kingdom:** Plantae
- **Division:** Tracheophyta
- **Subphylum:** Spermatophytina
- **Class:** Magnoliopsida
- **Order:** Solanales
- **Family:** Solanaceae
- **Genus:** *Capsicum*
- **Species:** *Capsicum annum*

(Sources: Beckles, 2012).

### **2.5.2.2 Ecological requirements of *Capsicum annum***

#### **2.5.2.2.1 Rainfall requirement**

Sweet pepper requires an average rainfall of 600mm per annum. Inadequate rainfall distribution during growth and development leads to blossom and rot disorder in pepper. Thus, during this period water stress result in fruit and flower abortion (Ashilenje, 2014).

#### **2.5.2.2 Soil requirement**

Pepper requires drained soil for growth, adequate organic matter, and optimum pH lies between 6.0 and 6.8. However, pepper adapts to growth under a wider pH range (pH 4.5 to 8.0). Pepper grows in sandy, clay sandy loam sandy soil. (Ashilenje, 2014).

#### **2.5.2.3 Temperature requirement**

The temperature required for adequate growth ranges from 18°C and 25°C. A temperature of 29.5°C was reported as optimum in pepper growth and development while a temperature below 18°C

inhibits the growth and development of fruit development. High temperatures (32°C) and low humidity lead to fruit and flower abortion during development (Ashilenje, 2014).

#### **2.5.2.4 Economic importance of *Capsicum annum***

Pepper is a vegetable crop. It belongs to the Solanaceae family. The Solanaceae family includes plants such as tomatoes, Irish potatoes, and egg plants, among others. Pepper is increasingly becoming important as a food, medicinal and industrial crop. Furthermore, the pungent nature of hot pepper renders it effective as a natural pest control product. Large amounts of pepper in developing countries are grown for export to the European Union and other markets. This contributes to foreign exchange earnings to the respective countries and income to farmers, majority of who are small scale growers (Ashilenje, 2014).

Pepper can be produced in the field and under greenhouse farming using conventional and organic procedures. Conventional production of pepper in the field is easily adoptable by farmers because of its productivity in the short run. However, organic production is of more benefits in the long run because its yields per unit area of land increase gradually while guaranteeing against chemical residues and environmental degradation. On the other hand, greenhouse production of pepper is capital intensive but profitable if good measures are taken to maintain sufficient crop nutrient supply as well as disease and pest free growing conditions (Ashilenje, 2014). (See Fig. 2.2)



**Fig 2.2: Sweet pepper plant (*Capsicum annuum*)**

**(Source: OECD, 2017)**

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 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 5°22'N and 5°19'N and longitudes, 6°58'E and 7°00'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 and night ranges from 18 and 24hrs, and the daily average temperature is 19°C to 28°C. (See Fig 3.1)

#### 3.2 Sources of planting materials

The two selected fruit crops used for the experiment which represent the experimental units are Tomato (*Solanum lycopersicum*) and sweet pepper (*Capsicum annum* Linn.). The choice of selection of the two fruit crops was based on their nutritional value, medicinal value, commercial and agronomic importance, as well as early maturity (Ogwu 2019). The seeds were purchased from the market; viability test was carried out and then grown in a screen-house at the Federal University of Technology Owerri. The crops were treated with distilled water for 3weeks, after which the application of acidulated rain water was carried out.

#### 3.3 Experimental design and treatments

##### 3.3.1 Planting Procedure (Method of Kausar *et al.*, 2019)

The experiment was laid in complete randomized design with the treatments (acidulated rain water) from Lagos, Port Harcourt, Kano and Gombe (4bags for each treatment). Five crops of tomato and pepper were planted in each bag. Each treatment was replicated 3 times (96 polythene bags) for the whole experiment. Three weeks after germination, each bag was sprayed with 500ml of collected rain water for six weeks.



Fig 3.1 Map of Federal University of Technology Owerri Showing Study Site (NIMET, 2016)

### **3.3.2 Collection of Rain water Samples (Method of Dinrifo *et al.*, 2010)**

Sampling of rainwater was carried out between April and July, 2022. Sampling was set up at open spaces in five locations (5 replicates) randomly selected to cover the built-up areas of the cities of Lagos, Port Harcourt, Kano and Gombe (control). A plastic container was placed on a 2m high platform. The sampling container was placed on the platform at the beginning of a rain event. After the rainfall, the container was removed, covered and stored. This was designed to eliminate significant contribution from dry deposition. Collected samples at the interval of two weeks were combined and analyzed.

### **3.3.3 Determination of pH and concentration of NO<sub>2</sub>, SO<sub>2</sub>, and CO<sub>2</sub> in rainwater**

#### **3.3.3.1 pH Test**

Rainwater sample (10ml) was placed in test tube and 20mls of bromothymol blue solution was added to each test tube. A glass electrode was dipped into the solution then dipped into the pH meter to test the pH of the water sample (Dinrifo, Babatunde, & Bankole, 2010).

#### **3.3.3.2 Sulphur dioxide (SO<sub>2</sub>)**

The spectrophotometric (DR2800 N0.290) method was used; whereby 10mL of water samples were poured into square sample cell, twelve drops of alkaline-Cyanide Reagent solution were then added to each cell to determine the level of the metals (Dinrifo *et al.*, 2010).

#### **3.3.3.3 Nitrogen dioxide (NO<sub>2</sub>)**

About five drops of water sample were poured in an evaporating dish, then two drops of diphenyl amine were added to sulphuric acid in a conical flask. The content of the flask was poured into the evaporating dish and the solution was heated. Blue colour indicated the presence of nitrate. The absorbance of the mixture was read on a spectrophotometer at 520nm (Dinrifo *et al.*, 2010).

### **3.3.3.4 Carbon (IV) dioxide (CO<sub>2</sub>) in rainwater**

Ten milliliters of rainwater sample were measured and four drops of phenolphthalein was added. The colorless solution was then titrated with 0.045 M of sodium carbonate solution. It was stirred gently with glass rod and sodium carbonate was added in bits. A faint pink color that remains for at least 30 seconds indicated the presence of carbon (IV) oxide. (Dinrifo *et al.*, 2010). The quantity of CO<sub>2</sub> was calculated as:

$$\text{CO}_2 (\mu\text{eq/L}) = \frac{\text{NaCO}_3 \times N \times 22 \times 1000}{\text{Volume of sample}}$$

**Where:** N = Normality of water, 22 = Equivalent rate of CO<sub>2</sub>

## **3.4 Collection of Experimental Data**

### **3.4.1 Determination of anatomical properties (Method of Tulay *et al.*, 2018)**

Leaf samples of 3 representative seedlings (n = 3) were used for anatomical study. Twenty-four hours after the last rain application, one leaf from each plant was taken. By applying the proper pressure, a leaf sample was fractured from the front and rear, exposing the lower surface's peel. The epidermal layer of the leaf's dorsal side was used to collect the leaf peel. A portion of a peel was transferred into a watch glass filled with water and then a few drops of safranin solution were added to stain the leaf peel. After five minutes, the stained peel was transferred to another watch glass containing water to wash off excess stain, and then transferred onto the slide containing a drop of glycerin (Tulay *et al.*, 2018). The slide was then covered with a cover slip. Using low power microscope (100X) the anatomy was observed.

### **3.4.2 Physiological assessment**

#### **3.4.2.1 Extraction and measurement of chlorophyll (Method of Harborne, 1987; Gu *et al.*, 2016)**

One gram of a fresh-cut leaf was ground using a pestle and mortar with 20–40ml of 80% acetone. The solution was centrifuged at 5000–10,000rpm for five minutes. Once the colorless residue was

produced, the supernatant was moved to another test tube and the process was repeated. The solution's absorbance was measured at 645 and 663nm wavelengths in comparison to a solvent (acetone) blank. Using spectrophotometer (DR2800 N0.290).

The amount of chlorophyll-A and B in the extract chlorophyll was measured using a spectrophotometer. 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:

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

$$\text{Chlorophyll a: } 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll b: } 22.9(A_{645}) - 4.68(A_{663})$$

**Where:**

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

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

### **3.4.3 Measurement of agro-morphological parameters (Method of Hunt 1990)**

#### **3.4.3.1 Shoot length**

The shoot length was measured using several factors. Using a tape ruler, the shoots' height was measured in cm, from the soil's surface to the plant's apex. The measurements were taken at intervals of 7days after the start of the application of acid rain treatment.

#### **3.4.3.2 Number of leaves and the area**

The leaf area (cm<sup>2</sup>) was obtained by using a ruler to measure the length of the leaf from the base to the tip. The leaf area was calculated by multiplying the length by the breadth and the leaf correction factor, 0.578 (Masarirambi *et al.*, 2012).

Leaf number was physically counted.

### 3.4.3.3 Stem girth length

From third week of seedling growth to last week, the stem girth was measured using a vernier caliper, and stem length (cm) was measured with measuring ruler from the ground level to the apical end of the shoot (Tongos, 2016).

### 3.4.3.4 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:

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

**Where:**

$W_2$  = 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

## 3.5 Statistical Analysis

The one-way analysis of variance (ANOVA) was used to analyze the data collected. The R v. 4.2.1 Statistical Package was used for all analyses. Duncan's multiple-range tests was used to assess the differences between means that were statistically significant ( $p < 0.05$ ).

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 Results

##### 4.1.1 pH and concentrations of acid derivatives (SO<sub>2</sub>, NO<sub>2</sub> and CO<sub>2</sub>)

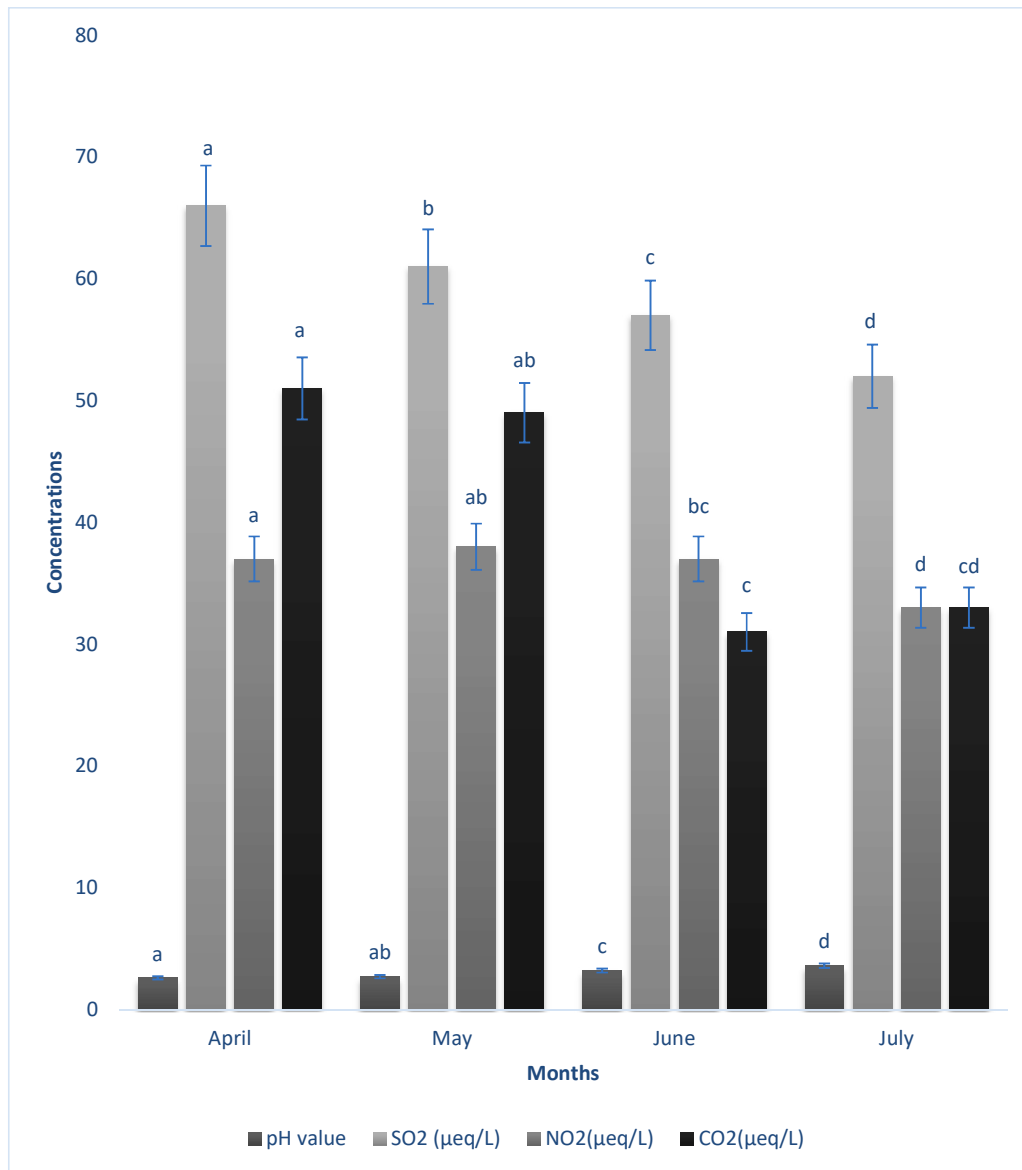
The results of the chemical proportion/concentration of the acid derivatives of the atmospheric acid gases for several months at Ikeja, Lagos State, are shown in (Fig. 4.1). An acid derivative (SO<sub>2</sub>) shows a significant difference at ( $p < 0.05$ ) in the month of April, May, June and July. However, CO<sub>2</sub> shows no significant difference between the month of April and May; and also, June and July at ( $p < 0.05$ ). Also, NO<sub>2</sub> did not differ significantly in the month of April, May and June, but differs in the month of July. The pH level did not differ in the month of April and May but differs in the month of June and July at ( $p < 0.05$ ).

Figure 4.2 shows the results of the chemical proportion/concentration of the acid derivative of atmospheric acid gases at Alesa Eleme, Port Harcourt, for various months. The acid derivative (SO<sub>2</sub>) shows significant difference in all the Months at ( $p < 0.05$ ). However, CO<sub>2</sub> did not differ in the month of April and May at ( $p < 0.05$ ) but differs significantly in the Month of June and July. While, NO<sub>2</sub> shows no significant difference in the month of April and May, but differs in the month of June and July at ( $p < 0.05$ ). The pH level did not differ in the month of April, May and June but differ in the month of July.

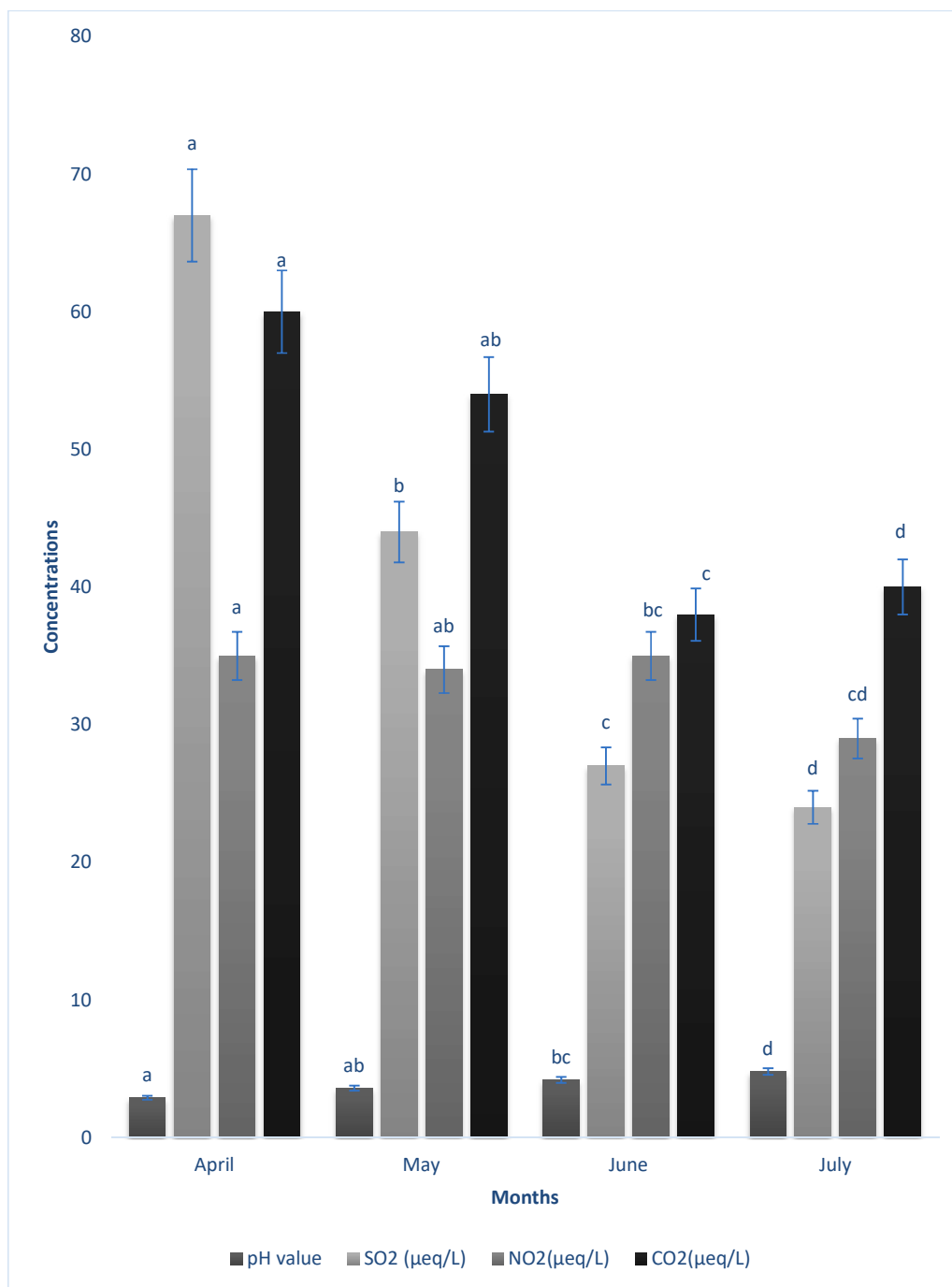
Figure 4.3 shows the chemical proportion/concentration of the acid derivative of atmospheric acid gases for several months along Sani Abacha Road in Kano State. An acid derivative SO<sub>2</sub> differs significantly in the month of April and July but did not differ in the month of May and June at ( $p < 0.05$ ). While, CO<sub>2</sub> shows no significant difference in the month of April, May, and June but differs in the month of July. Moreover, NO<sub>2</sub> did not differ between the month April

and May also between June and July significantly at ( $p < 0.05$ ). The pH level differs in the month of April and July but did not differ significantly in the month of May and June.

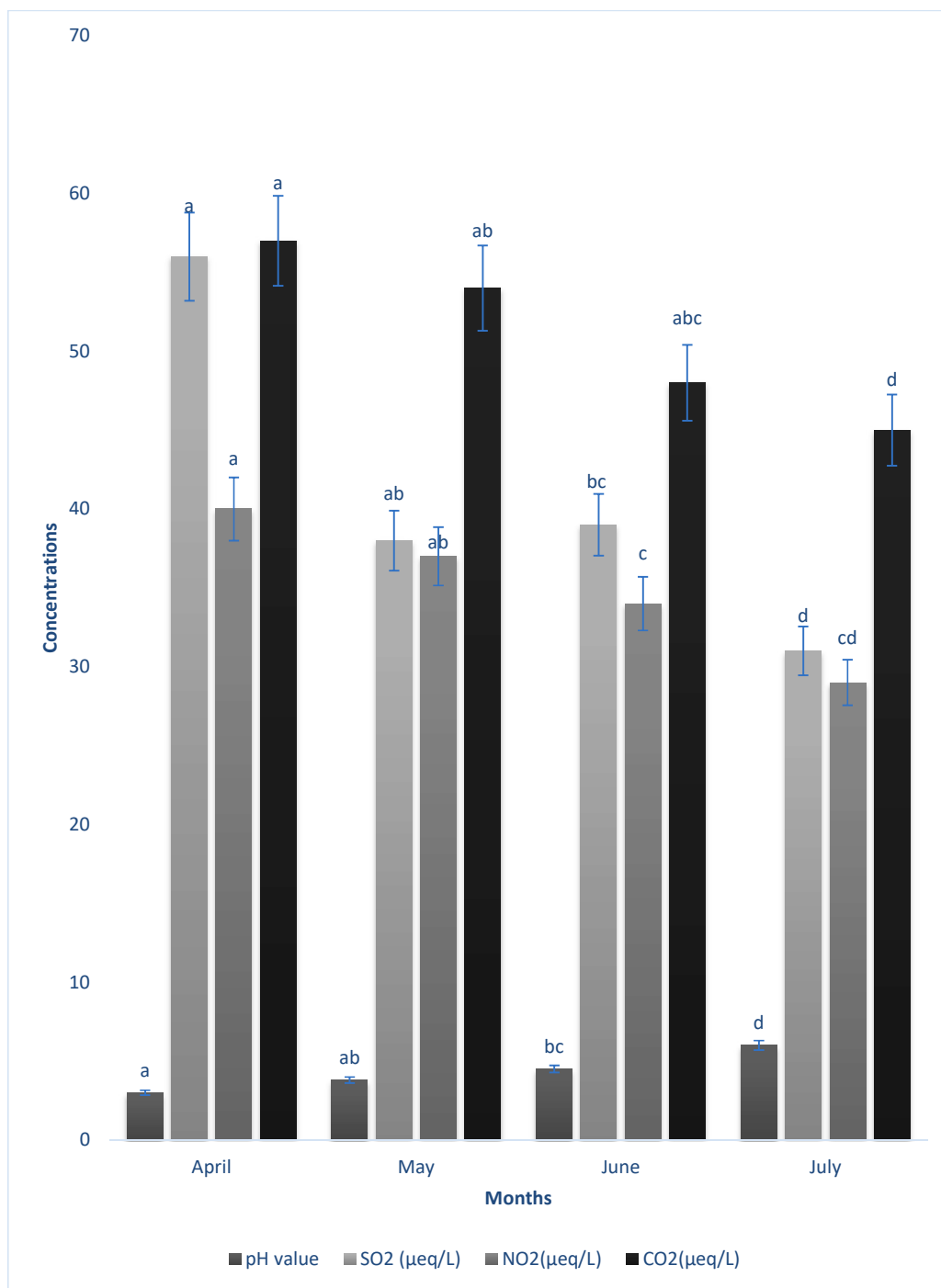
Figure 4.4 shows chemical proportion/concentration of the acid derivative of atmospheric acid gases for several months at Kwadon, Gombe State. An acid derivative  $\text{SO}_2$  differs significantly in all the months at ( $p < 0.05$ ).  $\text{NO}_2$  shows significant difference in the month April and May, and also in July but did not differ in the month of May and June. Moreover,  $\text{CO}_2$  differs significantly across the months. Furthermore, the pH level shows no significant difference at ( $p < 0.05$ ) across the months.



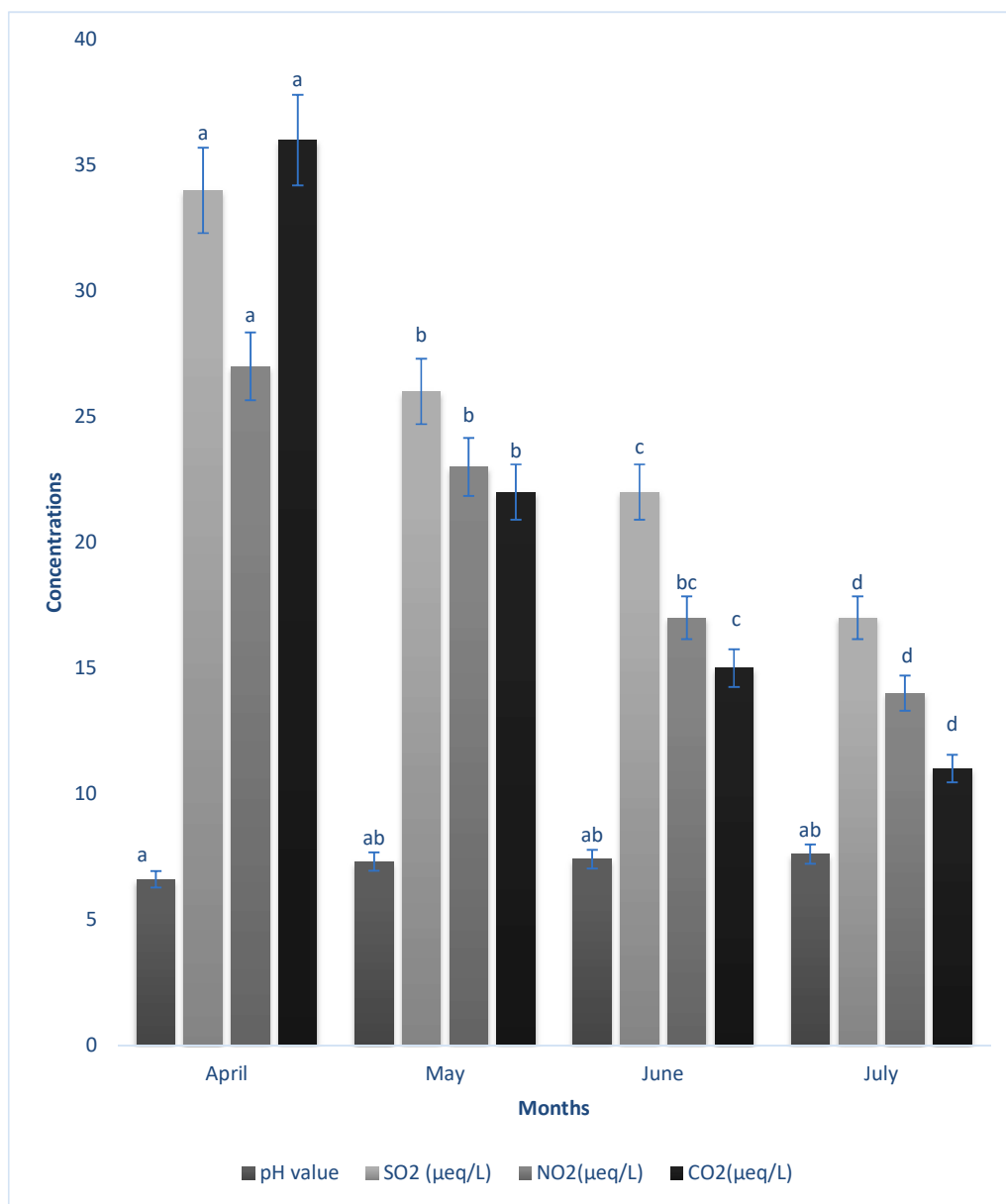
**Figure 4.1: Chemical composition of rain water collected from Ikeja, Lagos State.**



**Figure4.2: Chemical composition of rain water collected from Alesa Eleme, Port Harcourt State**



**Figure 4.3: Chemical composition of rain water collected at Sani Abacha Road, Kano State.**



**Figure 4.4: Chemical composition of rain water collected at Kwadon, Gombe State.**

### **4.1.2 Damages on leaf**

The morphology and anatomy of *Solanum lycopersicum* and *Capsicum annum* were affected by acidulated rain water. A thorough account of the physical damages brought on by acidulated rainwater in these two species was studied as follows.

#### **4.1.2.1 Visible leaf damage of simulated acid rain on leaves**

Plate 4.1 shows the morphological changes on *Solanum lycopersicum*. The rainwater harvested from Lagos and Port Harcourt caused physical damage of leaves such as formation of white scars and reddish color on the leaves. The changes were seen from the first application and continued to the last application. Rainwater harvested from Kano also caused morphological damage but not consistent. Fewer scars were seen on the control but no further damage.

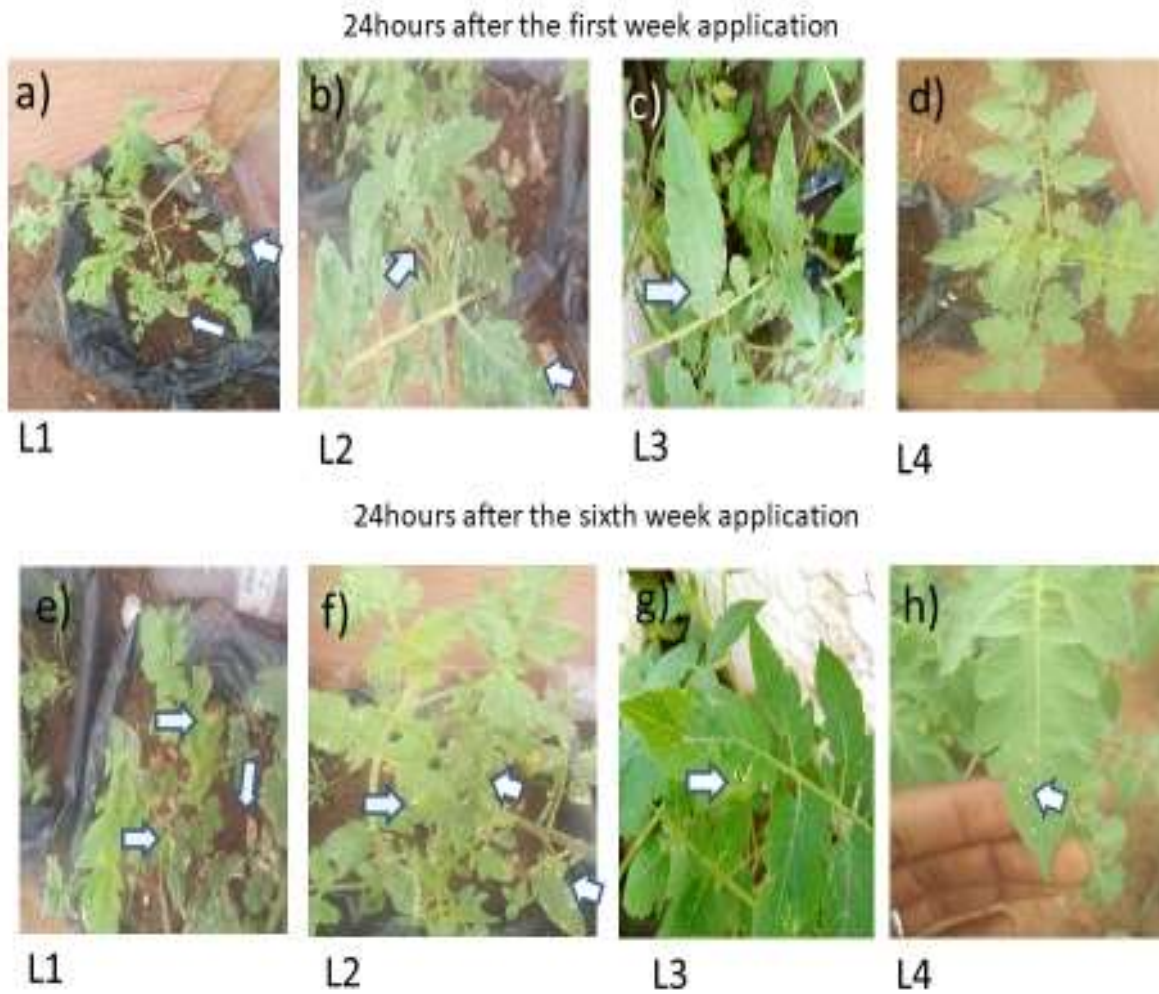
Plate 4.2 shows morphological changes on *Capsicum annum* including leaf damage, the development of white scars, and leaf drop. On leaves treated with rainwater from Lagos and Port Harcourt the changes were more pronounced. There were a few scars that formed in Kano, but there were none in Gombe (Control).

#### **4.1.2.2 Effect of low pH on Leaf anatomy**

Plate 4.3 shows the anatomical damages of the phylloplane. (Plate 4.3a) shows complete collapse of epidermal cells on the adaxial surface as well as the buildup of epicuticular waxes in specific intercostal regions. (Plate 4.3b) shows lobules of scarred tissue and necrosis. Also, abundant cellular contents are observed throughout the mesophyll which have an intense coloration after staining. (Plate 4.3c) shows intact mesophyll with fewer stomatal cells. (Plate 4.3d) reveals no damage on the mesophyll with numerous stomatal cells.

Plate 4.4 shows the phylloplane of *Capsicum annum*'s anatomical damages. Internally, there was total tissue damage in some areas of the margin, and the mesophyll of intercostal areas completely collapsed on the adaxial surface. (Plate 4.4a) shows the collapse of epidermal

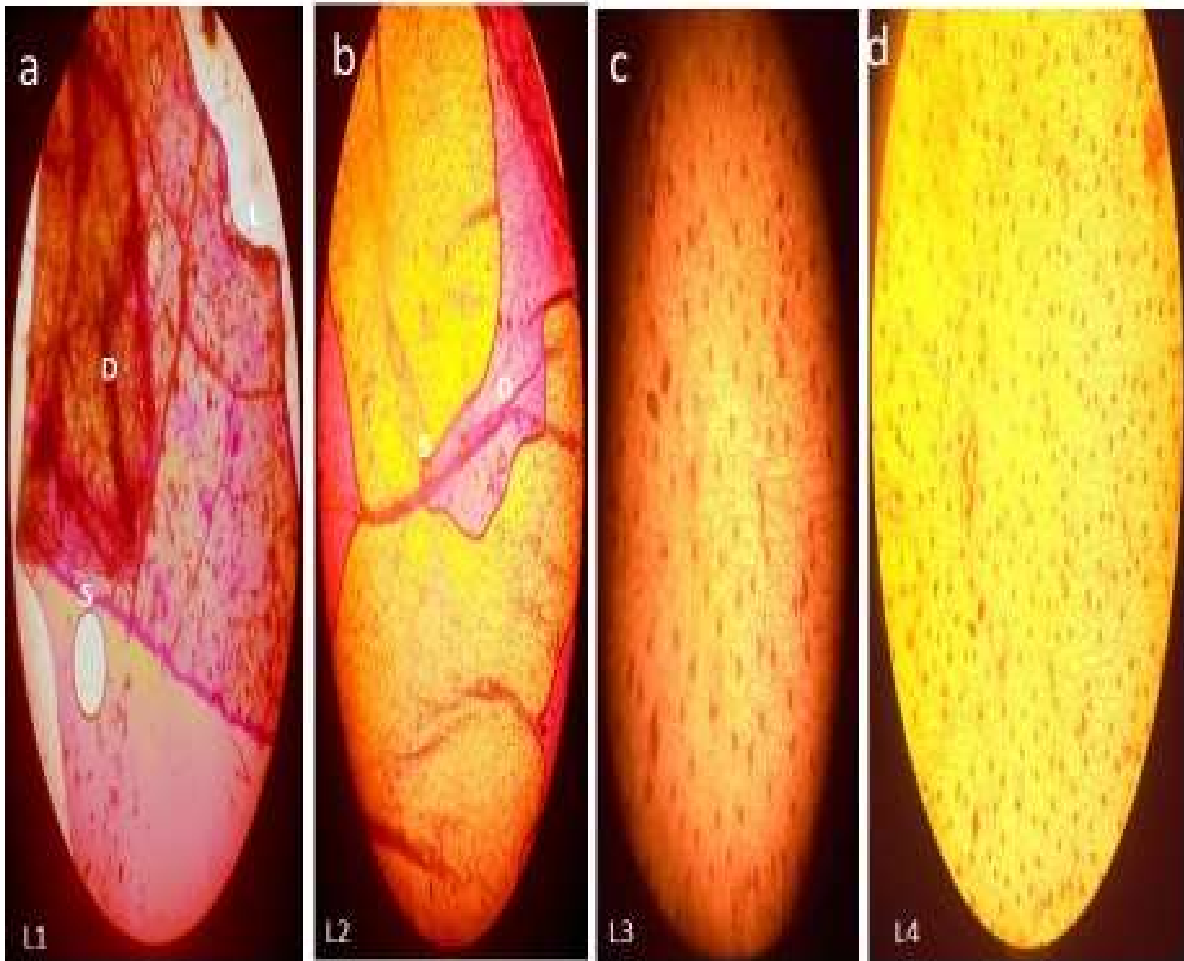
cells, while (Plate 4.4b) shows the modification of the cuticle. (Plate 4.4c) shows intact mesophyll with fewer scars tissues.



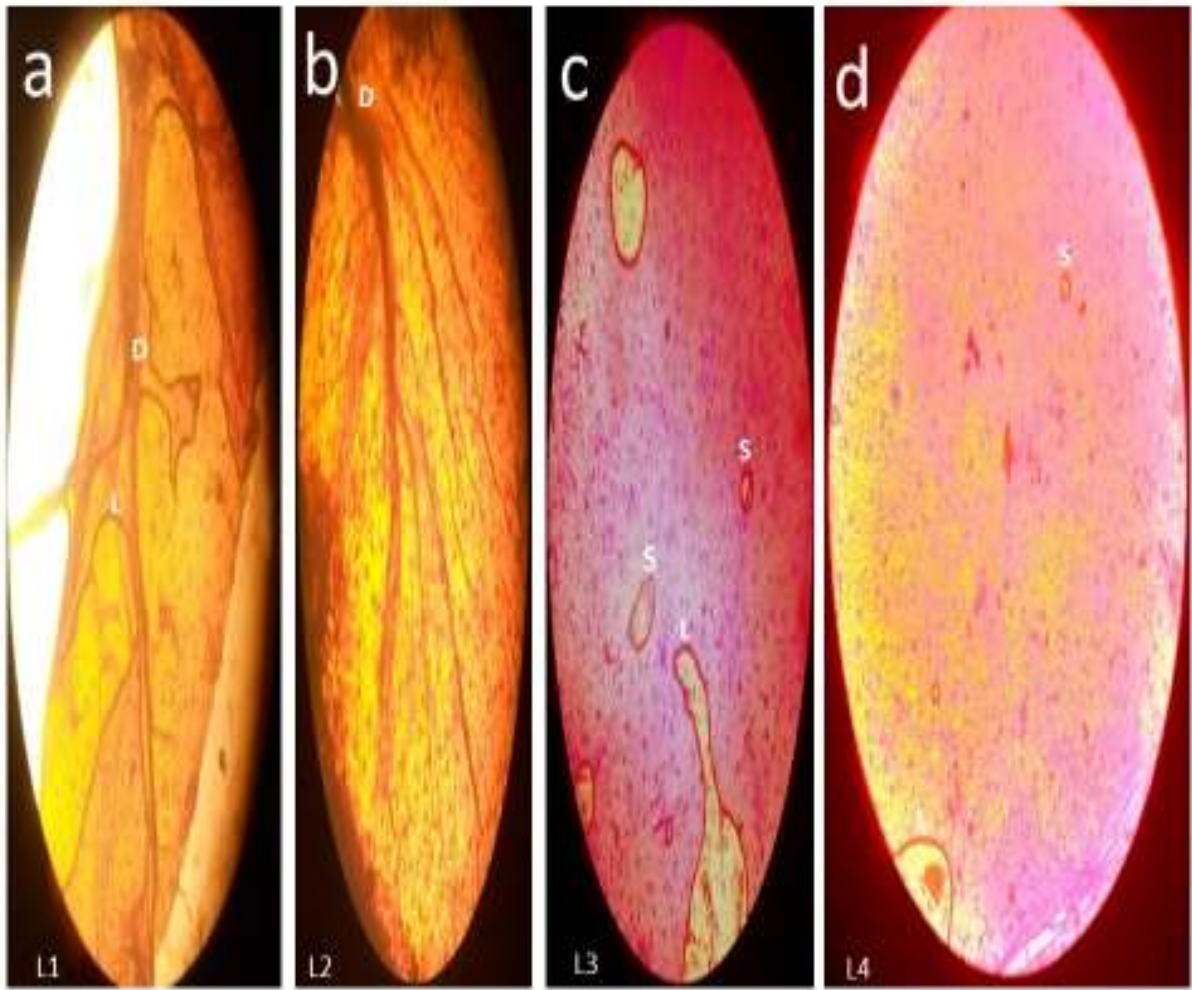
**Plate 4.1:** Visible damage of acidulated rain water on *Solanum lycopersicum*. (a-d) 24hours after the first week application of acidulated rain on leaves of *Solanum Lycopersicum*(e-h) 24hour after the sixth week application of acidulated rain on leaves of *Solanum Lycopersicum*. Arrows indicate some areas of damage caused by the sprayed acidic solution on the leaves. \*L indicate the locations in which the Rain water samples were collected. L1; Lagos/Ikeja.L2; Port Harcourt/Alesa Eleme.L3; Kano/Sani Abacha road.L4; Gombe/Kwadon.



**Plate 4.2:** Visible damage of acidulated rain water on *Capsicum annum*. (a-d) 24hours after the first week application of acidulated rain on leaves of *Capsicum annum*(e-h) 24hour after the sixth week application of acidulated rain on leaves of *Capsicum annum*. Arrows indicate some areas of damage caused by the sprayed acidic solution on the leaves. \*L indicates the locations in which the Rain water samples were collected. L1; Lagos/Ikeja.L2; Port Harcourt/Alesa Eleme.L3; Kano/Sani Abacha road.L4; Gombe/Kwadon.



**Plate4.3:** Anatomical changes in leaves of *Solanum Lycopersicum*. (a-d) adaxial view of the epidermis (100X). D: damage; S: scar; L: lobule.\*L indicate the locations in which the Rain water samples were collected. L1; Lagos/Ikeja. L2; Port Harcourt/Alesa Eleme. L3; Kano/Sani Abacha road.L4; Gombe/Kwadon.

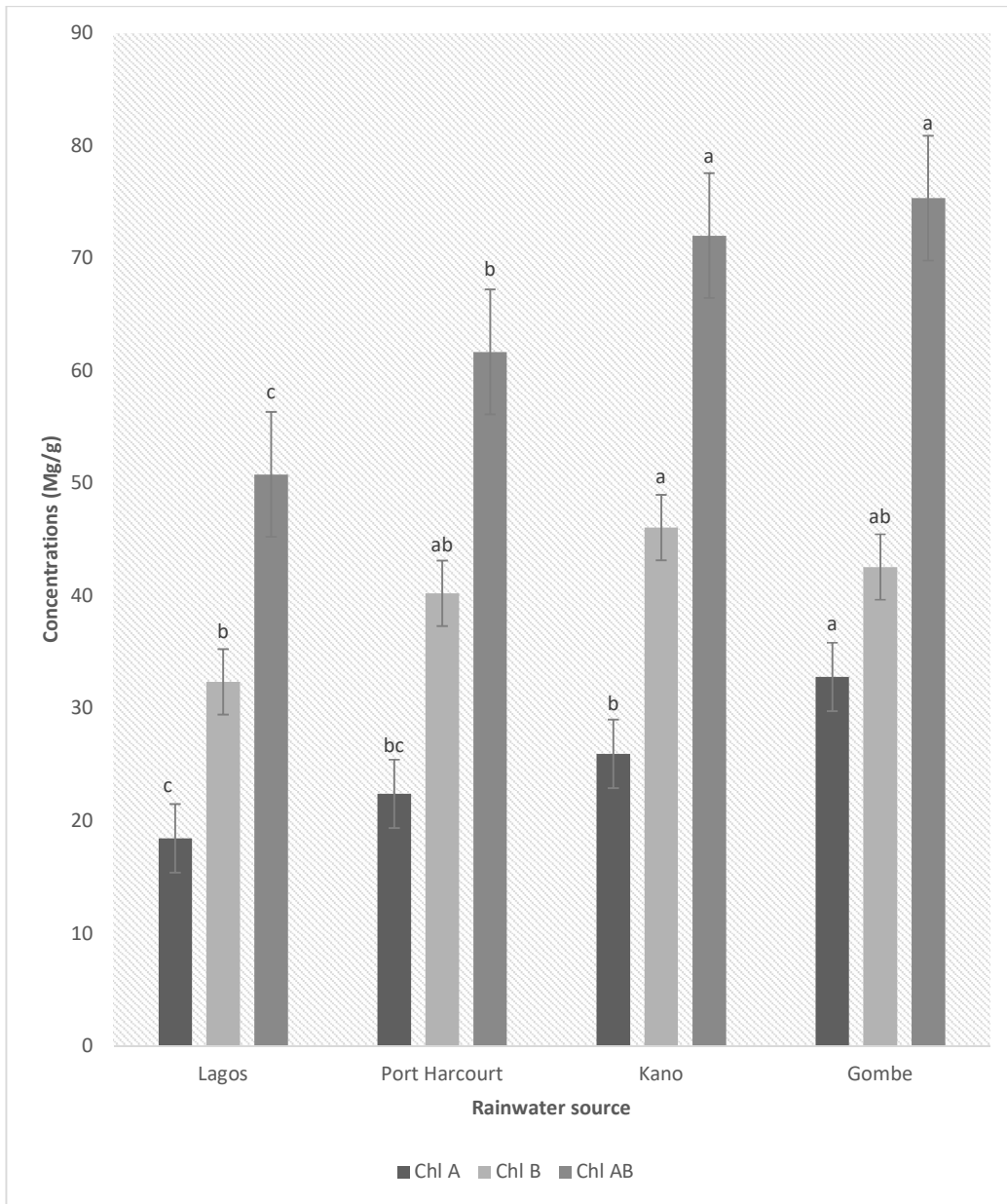


**Plate4.4:** Anatomical changes in leaves of *Capsicum annum*. (a-d) adaxial view of the epidermis (100X). D: damage; S: scar; L: lobule.\*L indicate the locations in which the Rain water samples were collected. L1; Lagos/Ikeja.L2; Port Harcourt/Alesa Eleme.L3; Kano/Sani Abacha road.L4; Gombe/Kwadon.

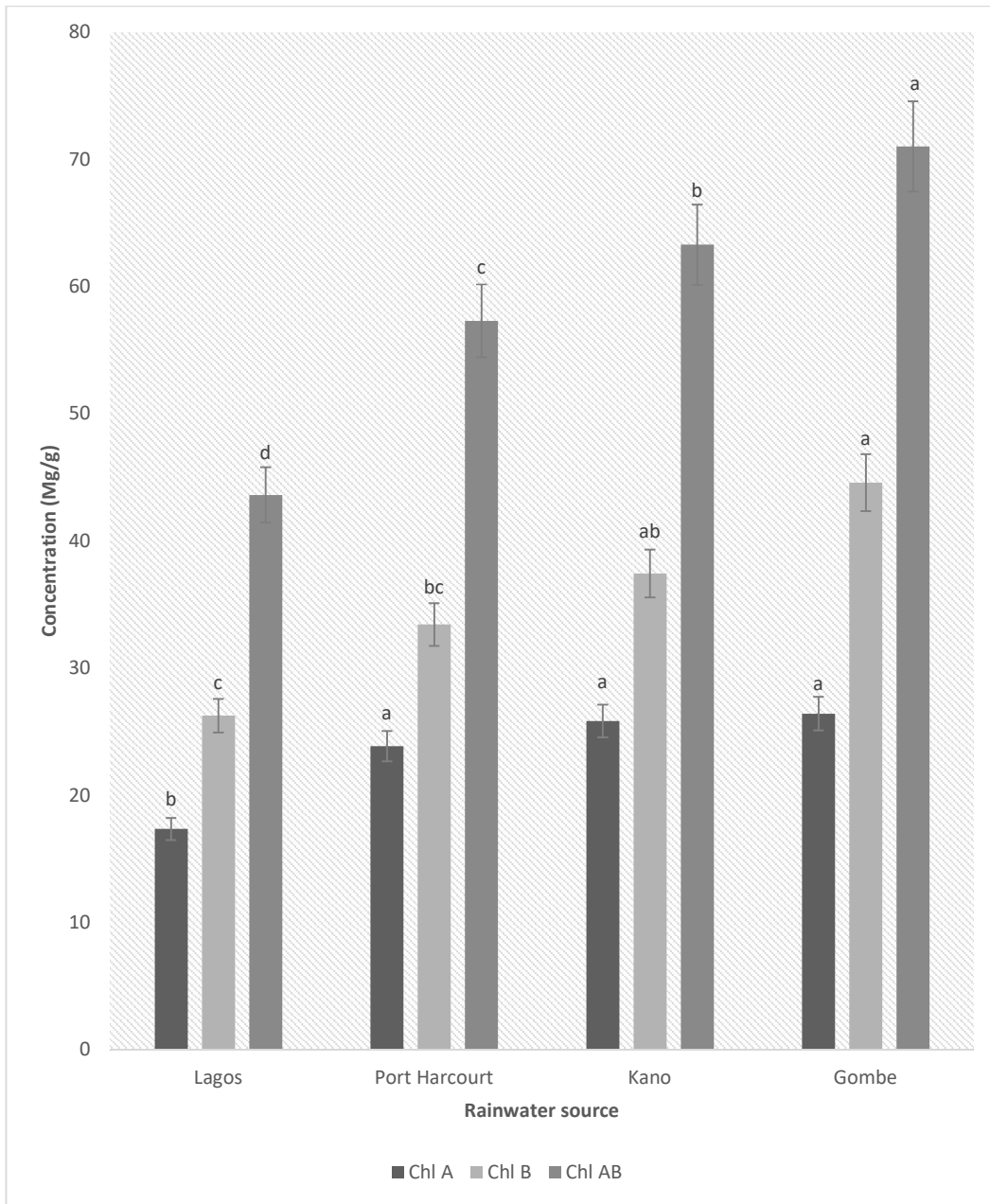
### 4.1.3 Chlorophyll content

Figure 4.5 shows the Chlorophyll A levels of *S. lycopersicum* irrigated with acidulated rainwater from; Lagos (18.45mg/g) and Port Harcourt (22.40mg/g) did not differ significantly. Again, that of Port Harcourt (22.40mg/g) and Kano (25.96mg/g) showed no difference significantly but that of Lagos and Kano showed significant difference. Moreover, rainwater from Gombe gave Chlorophyll A level of 32.79mg/g with a significant difference at ( $p < 0.05$ ) from the other treatments. All the plants irrigated with rainwater from; Lagos (32.35mg/g), Port Harcourt (40.22mg/g), Kano (46.06mg/g) and Gombe (42.56mg/g) did not differ significantly except that of Lagos and Kano which showed significant difference ( $p < 0.05$ ) in term of chlorophyll B content. In contrast, there was discernible difference in total chlorophyll at ( $p < 0.05$ ) for tomato plant irrigated with rainwater from Lagos (50.79mg/g) and Port Harcourt (61.67mg/g), but there was no significant difference for Kano (72.00mg/g) and Gombe (75.33mg/g).

The results from figure 4.6 shows that there was no difference in chlorophyll A concentration in plants irrigated with rainwater from Port Harcourt (23.88mg/g), Kano (25.86mg/g), and Gombe (26.44mg/g) at ( $p < 0.05$ ) but there was a significant difference in that of Lagos (17.37mg/g). The results also show that the quantities of chlorophyll B in plants irrigated with rainwater from; Lagos (26.27mg/g) and Port Harcourt (33.44mg/g), and also that of Kano (37.45mg/g) and Port Harcourt (33.44mg/g) were statistically similar, but considerably different between that of Lagos and Kano. Again, that of Gombe (44.59mg/g) significantly differed with that Lagos and Port Harcourt at ( $p < 0.05$ ) but similar with that of Kano. There was a statistically significant difference in total chlorophyll of plants irrigated with rainwater from; Lagos (43.63mg/g), Port Harcourt (57.30mg/g), Kano (63.29mg/g), and Gombe (71.02mg/g) at ( $p < 0.05$ ).



**Figure 4.5: Chlorophyll contents of *S. lycopersicum* Plants irrigated with Rainwater**



**Figure 4.6: Chlorophyll contents of *C. annuum* irrigated with Rainwater**

#### 4.1.4 Growth parameters

Table 4.1 shows agro-morphological parameters of *S. lycopersicum* irrigated with rainwater. The result reveals a significant difference in plant height ( $p < 0.05$ ) by rainwater from Lagos (16.81 cm), Port Harcourt (22.77cm), Kano (27.53cm), and Gombe (34.83cm). However, leaf area also showed significant difference ( $p < 0.05$ ) by rainwater from Lagos (5.177cm<sup>2</sup>), Port Harcourt (9.13cm<sup>2</sup>), Kano (12.99cm<sup>2</sup>) and Gombe (16.83cm<sup>2</sup>). Again, Stem girth also showed significant difference at ( $p < 0.05$ ) across the plants irrigated with rainwater from; Lagos (1.06cm), Port Harcourt (1.50cm), Kano (1.90cm) and Gombe (2.37cm). Furthermore, the leaf count showed significant difference ( $p < 0.05$ ) across the plants irrigated with rainwater from; Lagos (32.6), Eleme (47.7), Kano (66.6) and Gombe (79.5). Moreover, Relative growth recorded significant difference ( $p < 0.05$ ) with rainwater from Lagos recorded (0.33gg<sup>-1</sup>d<sup>-1</sup>), Port Harcourt (0.53gg<sup>-1</sup>d<sup>-1</sup>), Kano (0.70gg<sup>-1</sup>d<sup>-1</sup>) and Gombe (0.97gg<sup>-1</sup>d<sup>-1</sup>).

**Table4.2: Agromorphological parameters of *S. lycopersicum* irrigated with rainwater**

<b>Rainwater Source</b>	<b>Plant Height (cm)</b>	<b>Leaf area (cm<sup>2</sup>)</b>	<b>Stem girth (cm)</b>	<b>Number of leaves</b>	<b>RGR (gg<sup>-1</sup>d<sup>-1</sup>)</b>
<b>L1</b>	16.81 <sup>d</sup>	5.177 <sup>d</sup>	1.06 <sup>d</sup>	32.6 <sup>d</sup>	0.33 <sup>d</sup>
<b>L2</b>	22.77 <sup>c</sup>	9.13 <sup>c</sup>	1.50 <sup>c</sup>	47.7 <sup>c</sup>	0.53 <sup>c</sup>
<b>L3</b>	27.53 <sup>b</sup>	12.99 <sup>b</sup>	1.90 <sup>b</sup>	66.6 <sup>b</sup>	0.70 <sup>b</sup>
<b>L4 (Control)</b>	34.83 <sup>a</sup>	16.83 <sup>a</sup>	2.37 <sup>a</sup>	79.5 <sup>a</sup>	0.97 <sup>a</sup>

**Note:** L1; Lagos/Ikeja. L2; Port Harcourt/Alesa Eleme.L3; Kano/Sani Abacha Road.L4; Gombe/Kwadon.\*Typical value Using the Duncan's multiple-range test, the identical latter in a column for each parameter indicates no significant difference at ( $p < 0.05$ ).

Table 4.2 shows agro-morphological parameters of *C. annuum* irrigated with rainwater. The result showed significant difference on plant height of the plants irrigated with rainwater from Gombe (44.06cm) at ( $p < 0.05$ ) from the other treatments. However, the result showed

significant difference ( $p < 0.05$ ) on plants height irrigated with rainwater from; Lagos (24.64cm) and Kano (34.23cm). Conversely, it shows no significant difference in that of; Lagos (24.64cm) and Port Harcourt (32.50cm), Kano (34.23cm) and Port Harcourt (32.50cm). The Leaf area did not substantially differ with rainwater from; Lagos (7.45cm<sup>2</sup>) and Port Harcourt (8.31cm<sup>2</sup>) at ( $p < 0.05$ ). However, it did differ considerably with rainwater from Kano (12.82cm<sup>2</sup>) and Gombe (17.09cm<sup>2</sup>). The stem girth of plants irrigated with rainwater from; Lagos (2.00cm) and Port Harcourt (2.14cm), Kano (2.44cm) and Port Harcourt (2.14cm) revealed no significant difference at ( $p < 0.05$ ). However, Gombe (2.81cm) showed significant difference from the other treatments. In terms of leaf count, there was a substantial difference ( $p < 0.05$ ) in plants irrigated with rainwater from Lagos (38.6), Port Harcourt (66.2), Kano (97.1), and Gombe (123.7). For the relative growth, plants irrigated with rainwater from Lagos (1.73gg<sup>-1</sup>d<sup>-1</sup>) and Port Harcourt (2.03gg<sup>-1</sup>d<sup>-1</sup>) did not differ substantially at ( $p < 0.05$ ), while Kano (2.93gg<sup>-1</sup>d<sup>-1</sup>) and Gombe (4.15gg<sup>-1</sup>d<sup>-1</sup>) differed.

**Table 4.2: Agromorphological parameters of *C. annum* irrigated with rainwater**

<b>Rainwater Source</b>	<b>Plant Height (cm)</b>	<b>Leaf area (cm<sup>2</sup>)</b>	<b>Stem girth (cm)</b>	<b>Number of leaves</b>	<b>RGR (gg<sup>-1</sup>d<sup>-1</sup>)</b>
<b>L1</b>	24.64 <sup>c</sup>	7.45 <sup>c</sup>	2.00 <sup>c</sup>	38.6 <sup>d</sup>	1.734 <sup>c</sup>
<b>L2</b>	32.50 <sup>bc</sup>	8.31 <sup>c</sup>	2.14 <sup>bc</sup>	66.2 <sup>c</sup>	2.03 <sup>c</sup>
<b>L3</b>	34.23 <sup>b</sup>	12.82 <sup>b</sup>	2.44 <sup>b</sup>	97.1 <sup>b</sup>	2.93 <sup>b</sup>
<b>L4 (Control)</b>	44.06 <sup>a</sup>	17.09 <sup>a</sup>	2.81 <sup>a</sup>	123.7 <sup>a</sup>	4.15 <sup>a</sup>

**Note:** L1; Lagos/Ikeja. L2; Port Harcourt/Alesa Eleme.L3; Kano/Sani Abacha Road.L4; Gombe/Kwadon.\*Typical value Using the Duncan's multiple-range test, the identical latter in a column for each parameter indicates no significant difference at ( $p < 0.05$ ).

## 4.2 Discussion

The chemical composition in rainwater of the month of April from Ikeja and Alesa Eleme revealed significant levels of SO<sub>2</sub> (fig. 4.1 & 4.2), whereas high levels of CO<sub>2</sub> in rainwater from Sani Abacha Road, Kano, and Kwadon area of Gombe state were detected (fig. 4.3 & 4.4). Eleme, Kano and Gombe had a drop in the levels of SO<sub>2</sub>, CO<sub>2</sub>, and pH. This was likely caused by the fact that the majority of the accumulated contaminants must have been brought back to the surface by the frequent rainfall. However, in month of June, the CO<sub>2</sub> level in Ikeja and Eleme junction had the lowest concentration, whereas Sani Abacha Road, Kano, saw a rise. This is similar to the report of Burns *et al.*, (2016) who reported that most of the sources of acidic rain are the result of emissions from industrial activities, vehicles, and pollution formed from man's anthropogenic activities. However, these produced more air pollutants than naturally occurring sources of acidity, including sulfur, nitrogen, and carbon oxides (Chandra *et al.*, 2017).

The fluctuation of CO<sub>2</sub> in rainwater of areas with heavy industrial and vehicular activity reflects carbon emissions that add to the buildup of CO<sub>2</sub> in the atmosphere. Rainwater naturally contains acid due to atmospheric carbon dioxide dissolving in it (Carr & Neary, 2006). The levels of CO<sub>2</sub> could potentially be a result of the high volume of traffic. The majority of the pollution load is typically present in the first rush of rainwater that falls at the start of rainfall. The biggest contributor to these phenomena is the pollution buildup and deposition that occurs during dry seasons.

The morphological effects of acidity on the leaf surface as shown (Plate 4.1 & 4.2) reveals that acid rain had impact on the morphology, leaving white scars on the leaves. In the course of this study, the blotches on the leaves proliferated, spreading throughout the leaf and blurring the margins, stretched by acidulated rainwater from Ikeja/Lagos. The two species developed necrotic patches on the intercostal region and margins across the entire leaf surface at the conclusion of the trial. This could be as a result of high concentration of H<sub>2</sub>SO<sub>4</sub> and

HNO<sub>3</sub> in the rainwater which is synonymous with findings of Veronica *et al.*, (2020) and Pharm *et al.*, (2021). Visible leaf damages were present in all treatments but absent in the control of both test species. The effects of the acid rain on both plants were demonstrated to be dynamic, growing from the first application to the last application. This agrees with the results obtain by Sant' Anna-Santos *et al.*, 2006; Da Silva, *et al.*, 2005.

However, the leaves treated with acidulated rain water from Ikeja, the surface collapsed, the cuticle changed and the epidermal cells on the adaxial collapsed due to the acidulated rain water. When plants were treated with acidulated rainwater from Ikeja and Eleme, the cellular contents increased and the pin-colored staining of the mesophyll became more obvious. In addition, the damaged cells create scar tissue that gets quite black, separating it from the healthier tissue (Plates 4.3a & 4.4a). The tissue eventually engulfed the lobule, separating it from the rest of the leaf as it assumed an oval shape (Plate 4.3a). The epidermis completely collapsed in intercostal areas and along the edge of abaxial surfaces (Plate 4.3a), while epicuticular wax aggregation accumulated around the wounded sites. The mesophyll in several intercostal and margin zones partially or completely collapsed. The results of Veronica *et al.* (2020), Sant' Anna-Santos *et al.* (2006), and Da Silva *et al.* (2005) agree with these findings.

Plants performed best with the control (acidulated rain water from Kwadon/Gombe) when compared to other acidic treatments in terms of height, leaf area, stem girth, and the number of leaves (Table 4.1 & 4.2). The reduction in stem and girth length resulted from acidulated rain water is considered a reduced growth rate. The acidulated rain water reduces the growth of the plant and alters the function of the plasma membrane by increasing Al<sup>3+</sup> to a toxic concentration. Leaf area relatively increases photosynthesis. However, it increases the dry mass of the plant. When researching how simulated acid rain affected the growth of soya beans, pharm *et al.* (2021) came to a similar conclusion. They discovered that as the pH of the simulated acid rain dropped, stem height drastically fell. This is synonymous with the

conclusions of Vina *et al.* (2020), Han (2019), Liu *et al.* (2018), and Rani (2017). Again, it is also consistent with the findings of Pragati *et al.* (2016), Dursan *et al.* (2015), and Bridget *et al.* (2014).

In this study, chlorophyll levels dropped as acidity increased (Figure 4.5 & 4.6). The control (irrigated with acidulated rain water from Kwadon/Gombe) exhibits substantially more chlorophyll than other treatments. Reduced leaf area was the cause of the decrease in photosynthesis and chlorophyll concentrations. Information about the physiological condition of plants is provided by the chlorophyll content. Chlorophyll synthesis and chloroplast development were also negatively affected when exposed to acid rain. As a result, the amount of leaf chlorophyll is a crucial marker of direct foliage damage and has a direct impact on plant productivity, which is significantly decreased by acid rain. This has traditionally been explained by stating that because acid rain causes an imbalance in H<sup>+</sup> ions in leaf cells, the drop-in chlorophyll concentration may indicate their destruction. This suggests that physiological harm may exist even in the absence of morphological or anatomical damage. Similar results have been reported by Vina *et al.*, (2020), Kausar *et al.*, (2019), Shaukat *et al.*, (2018), and Long *et al.*, (2017).

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

The study established the benchmark for rainwater collected in Nigerian cities with high vehicular traffic and industrial activities. The actual information from the field reveals some intriguing insights: it demonstrates that emissions from vehicles as well as intensive industrial operations contributed more to the acidity of rainwater. It also revealed that rainwater that has been acidified has a significant impact on plants' morphology, anatomy, and physiology.

Anatomically, applying acidulated rainwater treatments to plants causes greatest harm to the epidermal cells, dissolving the cuticle, altering the permeability of the leaves, and causing necrosis. White scars, stunted growth, lesions, inhibition of leaf development, curving of leaves, withering of leaves, leaf abscission, and even death of plants are indicators of acid-poisoned plants in plants treated with acidulated rainwater, demonstrating that morphological damage led to anatomical harm. The anatomical damage was observed in Rain water collected from industrial areas such as Ikeja of Lagos State and Alesa Eleme of River State. Even at Sani Abacha Road where apparent damage was not present, the rainwater decreased the amount of chlorophyll a and b in both plant species, 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.

#### 5.2 Recommendations

It is recommended that phytoremediation through planting of trees; particularly acidophilic type should be done in the Cities of Lagos, Port Harcourt and Kano. Trees is known for its role of filtering atmospheric pollutant. Hence, they are called Lungs of the earth.

## CONTRIBUTIONS TO KNOWLEDGE

1. The study provides a comprehensive analysis of the acidulation of rainwater (April to July) in four different urban areas of Nigeria. The inclusion of atmospheric gases and their impact on plant anatomy and physiology adds depth to the research.
2. The rainwater samples collected over a span of four months, allowing for a thorough examination of seasonal variations and trends in acid derivatives.
3. The study highlights the significant impact of industrial and vehicular emissions on rainwater acidity. The observed alterations in plant anatomy and chlorophyll content provide valuable insights into the effects of acidified rainwater on vegetation.
4. The study's focus on acid rain and its detrimental effects on plants is highly relevant for regions facing industrial development and pollution. The recommendation to plant acidophilic trees to mitigate the effects of acid rain is a practical solution for environmental preservation.
5. The microscopic analysis of leaf anatomical damages resulting from acidulated rainwater is a crucial aspect of the research. The visual representation of damaged leaf structures enhances the understanding of the study's outcomes.
6. The study's findings have practical implications for sustainable development in acid rain-affected areas. The identification of acidophilic tree species can aid in planning urban green spaces resilient to acidic rainwater.
7. The study opens avenues for further research on the long-term effects of acidulated rainwater on plant ecosystems and the potential for implementing acidification mitigation strategies.
8. Overall, the study makes a valuable contribution to the understanding of acid rain's impact on vegetation in urban areas and emphasizes the importance of environmental conservation efforts in regions facing industrial development and pollution.

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# APPENDICES

## Appendix 1: Result of Elemental analysis of Rainwater



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### SNC/O RESULT OF ANALYSIS

DATE & TIME	4/26/2022 1:16:10 PM	P_ID	UMYU KYN
SAMPLE ID	4NI	USER ID	Administrator
QUANTITY (L)	1L	MODE	PSNC

#### SIGNALS

	L1	L2	L3	L4
pH value	2.6	2.9	3.0	6.6
Sulfur dioxide	66	67	56	34
Nitric dioxide	37	35	40	27
Carbon dioxide	51	54	57	36

BLANKS 5.4 57 28 30

KFACTORS 23.61 33.77 11.83 9.66

FILL	COMB	BOOST1	BOOST2
0	0	0	0

FILL TIME 30 SECONDS

**Model Name:** Series II CHNS/O Analyzer 2400  
**Serial No.:** 24IN9022721  
**Product Name:** Perkin Elmer

**ANALYST:** KABIR YAHUZA A/SLT  
 08106166111



**UMARU MUSA YAR'ADUA UNIVERSITY KATSINA**  
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**SNC/O RESULT OF ANALYSIS**

DATE & TIME 5/20/2022 12:16:06 PM P\_ID UMU KYN  
 SAMPLE ID 4NI USER ID Administrator  
 QUANTITY (L) 1L MODE PSNC

**SIGNALS**

	L1	L2	L3	L4
pH value	2.7	3.6	3.8	7.3
Sulfur dioxide	61	44	38	26
Nitric dioxide	38	34	37	23
Carbon dioxide	49	54	54	22

BLANKS 5.4 57 28 30

KFACTORS 23.61 33.77 11.83 9.66

FILL	COMB	BOOST1	BOOST2
0	0	0	0

FILL TIME 30 SECONDS

Model Name: Series II CHNS/O Analyzer 2400  
 Serial No.: 241N6022721  
 Product Name: Perkin Elmer

ANALYST: KABIR YAHUZA AISLT  
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**SNC/O RESULT OF ANALYSIS**

DATE & TIME	6/22/2022 12:11:23 PM	P_ID	UMYU KYN
SAMPLE ID	4NI	USER ID	Administrator
QUANTITY (L)	1L	MODE	PSNC

**SIGNALS**

	L1	L2	L3	L4
pH value	3.2	4.2	4.5	7.4
Sulfur dioxide	57	27	39	22
Nitric dioxide	37	35	34	17
Carbon dioxide	31	38	48	15

BLANKS 5.4 57 28 30

KFACTORS 23.61 33.77 11.83 9.66

FILL	COMB	BOOST1	BOOST2
0	0	0	0

FILL TIME 30 SECONDS

**Model Name:** Series II CHNS/O Analyzer 2400  
**Serial No.:** 241N6022721  
**Product Name:** Perkin Elmer

**ANALYST:** KABIR YAHUZA AISLT  
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**SNC/O RESULT OF ANALYSIS**

DATE & TIME 7/25/2022 12:39:20 PM P\_ID UMYU KYN  
 SAMPLE ID 4NI USER ID Administrator  
 QUANTITY (L) 1L MODE PSNC

SIGNALS

	L1	L2	L3	L4
pH value	3.6	4.8	6.0	7.6
Sulfur dioxide	52	24	31	17
Nitric dioxide	33	29	29	14
Carbon dioxide	33	40	45	11

BLANKS 5.4 57 28 30

KFACTORS 23.61 33.77 11.83 9.66

FILL	COMB	BOOST1	BOOST2
0	0	0	0

FILL TIME 30 SECONDS

Model Name: Series II CHNS/O Analyzer 2400  
 Serial No.: 24IN9022721  
 Product Name: Perkin Elmer

ANALYST: KABIR YAHUZA AISLT  
 08106166111

**Appendix 2:** Results of Statistical Analysis For Rain water

Analysis of Variance Tables for Rain water parameters from Lagos

Analysis of Variance Table

Response: Sulphur (IV) oxide

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
SO2	3	1423.268.78	7.4394	0.0002381	***
Residuals	24	2645.153.71			

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Nitrogen (IV) oxide

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
NO2	3	56.26564.7564.5865	0.0002146	0.0002146	***
Residuals	24	4.943275.603			

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Carbon (IV) oxide

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
CO2	3	54.8338.275	26.333	22.455e-07	***
Residuals	24	16.8112.134			

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: pH

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pH	3	4.6785	7.3412	20.5232	2.4653e-03 ***
Residuals	24	2.0345	6.975		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### DUNCAN'S MULTIPLE-RANGE POSTHOC TEST RAIN WATER

For Lagos

Means with the same letter are not significantly different.

Sulphur (IV) oxide

April	66	a
May	61	b
June	57	c
July	52	d

Means with the same letter are not significantly different.

Nitrogen (IV) oxide

April	37	ab
May	38	b
June	37	bc
July	33	d

Means with the same letter are not significantly different.

Carbon (IV) oxide

April	51	a
-------	----	---

May	49	ab
June	31	c
July	33	cd

Means with the same letter are not significantly different.

#### pH

April	2.6	a
May	2.7	a
June	3.2	b
July	3.6	d

#### For Port Harcourt

Means with the same letter are not significantly different.

#### Sulphur (IV) oxide

April	67	a
May	44	b
June	27	c
July	26	d

Means with the same letter are not significantly different.

#### Nitrogen (IV) oxide

April	35	a
May	36	ab
June	35	c
July	29	d

Means with the same letter are not significantly different.

Carbon (IV) oxide

April	60	ab
May	54	b
June	38	c
July	40	d

Means with the same letter are not significantly different.

pH

April	2.9	ab
May	3.6	b
June	4.2	bc
July	4.8	d

For Kano

Means with the same letter are not significantly different.

Sulphur (IV) oxide

April	56	a
May	38	bc
June	39	c
July	31	d

Means with the same letter are not significantly different.

Nitrogen (IV) oxide

April	40	ab
May	37	b
June	34	c
July	29	cd

Means with the same letter are not significantly different.

#### Carbon (IV) oxide

April	57	a
May	54ab	
June	48	ab
July	45	d

Means with the same letter are not significantly different.

#### pH

April	3.0	a
May	3.8	b
June	4.5	bc
July	6.0	d

#### For Gombe

Means with the same letter are not significantly different.

#### Sulphur (IV) oxide

April	34	a
May	26	b
June	22	c
July	17	bc

Means with the same letter are not significantly different.

Nitrogen (IV) oxide

April	27	ab
May	23	bc
June	17	c
July	14	d

Means with the same letter are not significantly different.

Carbon (IV) oxide

April	36	a
May	22	b
June	15	c
July	11	d

Means with the same letter are not significantly different.

pH

April	3.0	ab
May	3.8	b
June	4.5	ab
July	6.0	ab

### Appendix 3: Results of Statistical Analysis For Agromorphology and Chlorophylls

#### Analysis of Variance Tables for Tomato parameters except for chlorophyll

##### Analysis of Variance Table

Response: Height

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	1910.3	636.78	7.8894	0.0003587 ***
Residuals	36	2905.7	80.71		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

##### Analysis of Variance Table

Response: Stem\_girth

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	3.8628	1.28758	8.8174	0.0001625 ***
Residuals	36	5.2570	0.14603		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

##### Analysis of Variance Table

Response: Leaf\_area

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	594.83	198.275	16.341	7.235e-07 ***
Residuals	36	436.81	12.134		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

##### Analysis of Variance Table

Response: Number\_of\_leaves

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	40987	13662.2	22.482	2.242e-08 ***
Residuals	36	21877	607.7		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: RGR

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	35.496	11.8320	38.522	2.49e-11 ***
Residuals	36	11.057	0.3071		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Tables for Pepper parameters except chlorophyll

Analysis of Variance Table

Response: Height

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	1741.38	580.46	63.835	1.743e-14 ***
Residuals	36	327.35	9.09		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Stem\_girth

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	9.3827	3.12758	39.3	1.896e-11 ***

Residuals 36 2.8650 0.07958

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### Analysis of Variance Table

Response: Leaf\_area

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	754.47	251.489	60.965	3.479e-14 ***
Residuals	36	148.51	4.125		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### Analysis of Variance Table

Response: Number\_of\_leaves

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	12796.2	4265.4	34.265	1.198e-10 ***
Residuals	36	4481.4	124.5		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### Analysis of Variance Table

Response: RGR

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	2.22051	0.74017	40.646	1.194e-11 ***
Residuals	36	0.65557	0.01821		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Tables for Tomato chlorophyll alone

Analysis of Variance Table

Response: Chlorophyll\_A

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	555.63	185.210	8.4128	0.001387 **
Residuals	16	352.24	22.015		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Chlorophyll\_B

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	507.71	169.235	2.5368	0.09332 .
Residuals	16	1067.38	66.711		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Chlorophyll\_T

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	1843.54	614.51	11.411	0.0002994 ***
Residuals	16	861.67	53.85		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Tables for Pepper chlorophyll alone

Analysis of Variance Table

Response: Chlorophyll\_A

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	259.44	86.479	4.6029	0.01661 *
Residuals	16	300.60	18.788		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Chlorophyll\_B

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	879.06	293.021	7.8154	0.001955 **
Residuals	16	599.89	37.493		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: Chlorophyll\_T

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rain water	3	2008.66	669.55	36.746	2.094e-07 ***
Residuals	16	291.54	18.22		

---

Signif.codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

DUNCAN'S MULTIPLE-RANGE POSTHOC TEST FOR TOMATO

For Tomato parameters except chlorophyll

Means with the same letter are not significantly different.

Height groups

L4	44.06	a
L3	34.23	b
L2	32.50	bc
L1	24.64	c

Means with the same letter are not significantly different.

#### Stem\_girth groups

L4	2.81	a
L3	2.44	b
L2	2.14	bc
L1	2.00	c

Means with the same letter are not significantly different.

#### Leaf\_area groups

L4	17.096	a
L3	12.820	b
L2	8.319	c
L1	7.459	c

Means with the same letter are not significantly different.

#### Number\_of\_leaves groups

L4	123.7	a
L3	97.1	b
L2	66.2	c
L1	38.6	d

Means with the same letter are not significantly different.

RGR groups

L4	4.158	a
L3	2.931	b
L2	2.038	c
L1	1.734	c

For Chlorophyll content Tomato

Means with the same letter are not significantly different.

Chlorophyll\_A groups

L4	32.7920	a
L3	25.9654	b
L2	22.4064	bc
L1	18.4588	c

Means with the same letter are not significantly different.

Chlorophyll\_B groups

L4	46.0666	a
L3	42.5650	ab
L2	40.2256	ab
L1	32.3532	b

Means with the same letter are not significantly different.

Chlorophyll\_T groups

L4	75.3324	a
L3	72.0068	a
L2	61.6744	b
L1	50.7942	c

DUNCAN'S MULTIPLE-RANGEPOSTHOC TEST FOR PEPPER

Chlorophyll contents alone

Means with the same letter are not significantly different.

Chlorophyll\_A groups

pH5.6	26.4412	a
pH3.5	25.8624	a
pH3.0	23.8860	a
pH2.5	17.3718	b

Means with the same letter are not significantly different.

Chlorophyll\_B groups

L4	44.5956	a
L3	37.4508	ab
L2	33.4422	bc
L1	26.2774	c

Means with the same letter are not significantly different.

Chlorophyll\_T groups

L4	71.0202	a
L3	63.2916	b
L2	57.3090	c
L1	43.6342	d

Other parameters for Pepper

Means with the same letter are not significantly different.

Height groups

L4	34.83	a
L3	27.53	b
L2	22.77	c
L1	16.81	d

Means with the same letter are not significantly different.

Stem\_girth groups

L4	2.37	a
L3	1.90	b
L2	1.50	c
L1	1.06	d

Means with the same letter are not significantly different.

Leaf\_area groups

L4	16.838	a
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L3	12.999	b
L2	9.138	c
L1	5.177	d

Means with the same letter are not significantly different.

Number\_of\_leaves groups

L4	79.5	a
L3	66.6	b
L2	47.7	c
L1	32.6	d

Means with the same letter are not significantly different.

RGR groups

L4	0.974	a
L3	0.708	b
L2	0.532	c
L1	0.333	d

## Appendix 4: Agro-morphology and Physiological Data of Tomato recorded.

Parameters	pH	Tomato												
		Period	Replicates											
			R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
Plant_heights	Lagos	Week <sub>0</sub>	6.4	5.5	7	7.5	5.5	4.3	5.2	6.3	6.8	7.8		
		Week <sub>2</sub>	12	14.5	11.5	8.5	10.1	15	11.4	8.7	11.5	10.2		
		Week <sub>4</sub>	15	24	20	15	21.5	34.5	11	20	15	13.5		
		Week <sub>6</sub>	18.4	16	20	20.8	20	35.1	18.4	18.6	38.6	40.5		
		P/Harcourt	Week <sub>0</sub>	6.3	5.5	5.8	5.3	5.4	6	5.1	4.8	5.8	5	
			Week <sub>2</sub>	12.5	14.2	16.1	12	10.8	9.5	11.5	10.5	8.3	8.5	
	Week <sub>4</sub>		25	20.5	21.5	27	21	29	20	30	34.5	20.5		
	Week <sub>6</sub>		25	49.2	44.5	30.8	32	27	25.4	31	33.4	26.7		
	Kano		Week <sub>0</sub>	6.3	5.5	4.5	6.7	4.4	5.1	4.5	5.5	6.3	5.8	
			Week <sub>2</sub>	12.1	15	13	12	14	13.6	16	10.1	12	14.3	
		Week <sub>4</sub>	33.4	21.5	20	20	25	25.5	30.5	31.5	32.5	30		
		Week <sub>6</sub>	30	38.8	21.2	28.3	40.1	31.5	43.5	26.3	55	27.6		
		Gombe	Week <sub>0</sub>	7.2	6.8	4.8	5.5	5.5	5.4	6.3	6.3	6.5	6.4	
			Week <sub>2</sub>	10.1	12.5	13	14	16.5	17	11.5	23	15.5	14.4	
	Week <sub>4</sub>		37	42.5	24.5	22	28.2	32	11	32.5	32	35.5		
	Week <sub>6</sub>		50.2	44	38	25.5	44.5	53	45.3	40	51.6	48.5		
	Stem_girth		Lagos	Week <sub>0</sub>	0.8	1	1.5	0.8	1	0.5	0.4	0.5	0.9	0.8
				Week <sub>2</sub>	0.9	1.1	1.9	1	1.2	0.7	0.5	0.9	1.1	1
		Week <sub>4</sub>		1.2	1.8	1.8	1.1	1.5	1.4	1	2	1.3	1.3	
		Week <sub>6</sub>		2.3	1.9	2.5	2.1	2	1.8	1.3	1.5	2.4	2.2	
		P/Harcourt		Week <sub>0</sub>	0.7	1.1	1.2	1.3	0.8	0.4	1.2	0.8	0.8	0.9
				Week <sub>2</sub>	1.5	1.4	2	1.5	1.8	1	1.5	1.5	1.6	1.4
			Week <sub>4</sub>	1.6	1.5	2.5	1.8	1.8	2	1.8	2.2	1.8	2.1	
			Week <sub>6</sub>	2	2.1	2	1.8	2	2.5	2.5	2.1	1.8	2.6	
Kano			Week <sub>0</sub>	1.1	1.8	1.2	1	1	1.5	0.8	1	1	1.4	
			Week <sub>2</sub>	2.2	2.5	1.8	1.8	1.7	2	1.4	1.8	1.7	1.5	
		Week <sub>4</sub>	2.7	2.3	2.5	1.8	2.1	2.2	2.1	2.1	2.2	2		
		Week <sub>6</sub>	2.4	1.9	2	2.7	3	1.9	2.6	2.8	2.3	2.8		
		Gombe	Week <sub>0</sub>	1.3	0.6	1.1	0.5	1.5	0.8	1	1	1	1.5	
			Week <sub>2</sub>	2.6	2.8	2.2	2	2	1.8	2.4	1.7	2.2	2	
Week <sub>4</sub>			2.5	2.4	2.2	2.5	2.3	2	2.2	2.6	3	3.2		
Week <sub>6</sub>			3	3.2	2.8	2.6	2.1	2.6	3.5	2.5	2.5	3.3		
Leaf_Area			Lagos	Week <sub>0</sub>	0.84	2.01	2.01	1.55	2.53	1.45	1.88	2.11	6.83	6.31
				Week <sub>2</sub>	1.04	2.31	1.88	3.51	3.47	2.35	2.02	2.48	9.16	13.4
		Week <sub>4</sub>		3.78	3.44	5.34	4.86	2.87	5.21	3.23	3.44	15.77	15.17	
		Week <sub>6</sub>		3.54	3.45	5.55	4.47	3.43	4.83	6.11	5.65	17.23	20.33	
		P/Harcourt		Week <sub>0</sub>	2.5	2.32	2.59	1.11	2.96	3.11	5.02	4.5	1.59	2.12
				Week <sub>2</sub>	2.59	2.91	3.12	1.14	3.52	3.32	5.09	8.7	2.53	2.71
			Week <sub>4</sub>	4.46	5.55	5.98	6.49	6.75	5.12	7.14	9.47	5.65	4.54	
			Week <sub>6</sub>	9.12	13.45	10.25	6.55	8.55	5.56	7.22	10.35	6.11	6.03	
	Kano		Week <sub>0</sub>	8.65	4.53	5.54	5.78	5.54	3.55	5.57	3.56	5.04	3.54	
			Week <sub>2</sub>	10.12	7.77	4.97	6.31	6.36	4.58	6.21	7.58	5.13	5.21	
		Week <sub>4</sub>	10.56	10.72	7.97	11.33	10.56	8.36	10.22	8.74	8.9	9.18		
		Week <sub>6</sub>	11.33	12.5	13.22	13.44	11.05	15.11	13.64	13.74	10.12	14.05		
		Gombe	Week <sub>0</sub>	6.31	5.58	6.83	10.15	4.56	2.56	4.5	4.23	4.09	5.5	
			Week <sub>2</sub>	13.4	9.55	9.16	14.42	9.72	6.25	8.58	7.21	14.31	8.67	
	Week <sub>4</sub>		15.17	13.15	15.77	10.58	11.1	16.26	15.02	15.17	13.53	11.47		
	Week <sub>6</sub>		20.33	18.33	17.23	15.25	16.86	18.71	17.45	15.68	15.5	15.62		
	Number of leaf		Lagos	Week <sub>0</sub>	10	11	8	9	13	12	12	11	10	11
				Week <sub>2</sub>	13	17	13	18	14	13	11	13	11	14
		Week <sub>4</sub>		32	35	27	21	31	28	26	18	13	25	
		Week <sub>6</sub>		33	45	43	24	54	38	31	43	34	41	
		P/Harcourt		Week <sub>0</sub>	7	8	13	11	11	15	13	11	12	13
				Week <sub>2</sub>	17	17	21	12	19	19	18	20	21	17
			Week <sub>4</sub>	69	56	48	51	31	56	31	29	45	55	
			Week <sub>6</sub>	78	98	73	79	46	68	45	43	68	64	
Kano			Week <sub>0</sub>	8	11	9	11	6	17	10	13	8	13	
			Week <sub>2</sub>	12	16	27	20	22	25	19	28	25	26	
		Week <sub>4</sub>	65	46	45	66	55	65	66	70	73	86		
		Week <sub>6</sub>	99	99	81	78	93	103	121	111	102	84		
		Gombe	Week <sub>0</sub>	11	8	11	11	11	16	15	8	12	9	
			Week <sub>2</sub>	20	12	18	13	19	32	22	26	79	34	
Week <sub>4</sub>			96	88	52	66	82	66	189	32	64	83		
Week <sub>6</sub>			129	180	94	156	207	88	103	81	105	94		
Chlorophyll			Lagos	A	15.253	28.438	16.246	16.304	16.053					
				B	35.852	23.326	35.881	15.945	50.762					
		TOTAL		51.087	51.748	52.108	32.238	66.79						
		P/Harcourt		A	16.56	27.987	21.238	28.312	17.935					
				B	40.037	42.574	40.037	33.494	44.986					
				TOTAL	56.576	70.537	56.576	61.786	62.897					
			Kano	A	26.173	27.483	25.726	26.328	24.117					
				B	43.221	46.102	46.55	46.143	48.317					
	TOTAL			69.37	71.803	74.008	72.446	72.407						
	Gombe	A		34.294	27.648	35.751	27.573	38.694						
		B		42.353	52.799	30.884	46.824	39.965						
		TOTAL		76.623	80.419	66.615	74.371	78.634						
		Relative growth	Week <sub>6</sub>	Lagos	1.5	1.4	2.22	1.13	1.55	1.58	1.88	1.37	2.29	2.42
				P/Harcourt	1.16	1.56	3.1	1.99	2.5	1.76	1.5	1.82	2.56	2.43
				Kano	3.11	2.9	2.75	3.06	2.61	4.11	2.56	2.11	2.61	3.49
	Gombe			3.82	3.52	3.8	4.66	4.08	3.69	4.3	4.55	5.49	3.67	

Appendix 5: Agro-morphology and Physiological Data of Pepper recorded.

Parameters	pH	Period	pepper											
			Replicates											
			R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
Plant_heights	Lagos	Week <sub>0</sub>	6.4	11.5	5.8	6.9	8.5	6.3	8	7.4	7.9	6		
		Week <sub>2</sub>	10.5	12	11.3	8.4	10	10.5	12.3	10	9.5	6.8		
		Week <sub>4</sub>	16	17.4	11.8	10.2	11.6	12.6	17	14	14.4	16.4		
		Week <sub>6</sub>	19	19	13.3	14	12.4	14.8	20.4	16.7	18.2	20.3		
		Week <sub>0</sub>	8.5	11.5	7.5	7.9	7	6.5	7.5	7.2	7.4	7.5		
		Week <sub>2</sub>	12.7	14.5	12.6	11.6	12.4	8.7	11.1	12.4	12	13		
	P/Harcourt	Week <sub>4</sub>	18.5	18.6	20.2	16	18	12.6	17	20.5	14.6	17.8		
		Week <sub>6</sub>	22.5	23.5	25.7	20.3	26.2	19.5	25	23	21.2	20.8		
		Week <sub>0</sub>	4.5	7.8	6.5	11.5	5.5	7.6	6.3	11.2	8.2	8.1		
		Week <sub>2</sub>	11.5	12.5	12.3	12.5	10	12.1	13.4	13	12.4	13.3		
		Week <sub>4</sub>	22.4	25	18.2	16.5	17.4	22.1	20.1	19.5	22.2	25		
		Week <sub>6</sub>	29.5	27.4	26	20.4	28.5	28	29.1	27	29	30.4		
	Kano	Week <sub>0</sub>	8.6	6.8	11.5	7.5	7.8	8.2	6.8	6	6.5	6		
		Week <sub>2</sub>	14.8	14	13.5	11.8	13.6	12.4	11.5	13.5	12.7	13		
		Week <sub>4</sub>	26.5	29	25.5	27.8	27.5	21.7	19.4	32	22	29.6		
		Week <sub>6</sub>	35.5	38.6	33	36.5	39.8	29.6	28.2	34	35.3	37.8		
		Week <sub>0</sub>	0.3	0.3	0.2	0.2	0.4	0.3	0.2	0.4	0.4	0.3		
		Week <sub>2</sub>	0.6	0.5	0.4	0.5	0.6	0.5	0.5	0.5	0.6	0.5		
Stem_girth	Lagos	Week <sub>4</sub>	0.9	0.5	0.7	0.8	0.7	0.5	0.6	1	1	1.7		
		Week <sub>6</sub>	1.2	0.8	0.8	1.1	0.9	0.7	0.9	1.2	1.2	1.8		
		Week <sub>0</sub>	0.3	0.4	0.2	0.2	0.2	0.3	0.4	0.4	0.3	0.4		
		Week <sub>2</sub>	0.8	0.6	0.7	0.5	0.8	0.5	0.7	0.6	0.6	0.8		
		Week <sub>4</sub>	1.4	1.6	0.8	1.5	0.9	0.8	1.5	0.9	1	1.5		
		Week <sub>6</sub>	1.4	1.6	1.1	1.5	1.1	1.6	1.8	1.5	1.4	2		
	P/Harcourt	Week <sub>0</sub>	0.5	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.4	0.2		
		Week <sub>2</sub>	1	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.6		
		Week <sub>4</sub>	1.7	1.1	1.5	1	1.7	1.4	1.3	1.5	1.5	1.8		
		Week <sub>6</sub>	2.4	1.9	1.9	1.9	2	1.6	1.7	1.8	1.9	1.9		
		Week <sub>0</sub>	0.5	0.2	0.2	0.3	0.3	0.4	0.2	0.4	0.4	0.4		
		Week <sub>2</sub>	1	0.8	1	0.8	0.8	0.9	0.9	0.8	0.8	0.9		
	Kano	Week <sub>4</sub>	1.8	2.1	1.3	2.5	1.8	1.6	1.9	1.6	1.7	1.8		
		Week <sub>6</sub>	2.4	2.5	2	2.7	1.9	2.4	2.5	2	2.5	2.8		
		Week <sub>0</sub>	2.65	3.33	2.6	3.45	3.98	3.64	1.8	2.6	3.55	4.11		
		Week <sub>2</sub>	3.42	3.89	3.67	3.98	5.69	4.37	2.42	3.65	3.91	4.67		
		Week <sub>4</sub>	4.23	4.12	4.76	4.67	5.92	4.69	3.18	4.46	4.32	4.89		
		Week <sub>6</sub>	4.51	4.95	5.5	5.37	6.34	4.9	3.82	5.12	4.84	6.42		
Leaf_Area	Lagos	Week <sub>0</sub>	3.66	1.8	4.12	4.05	2.4	4.11	4.05	2.33	1.8	1.83		
		Week <sub>2</sub>	5.43	3.78	4.78	4.87	4.21	8.32	4.91	4.87	4.23	3.54		
		Week <sub>4</sub>	7.38	8.67	6.57	7.45	4.95	11.21	10.11	5.65	7.56	4.87		
		Week <sub>6</sub>	7.87	8.98	7.11	7.87	5.64	14.5	12.42	10.14	9.4	7.45		
		Week <sub>0</sub>	2.83	2.64	3.64	3.65	3.76	2.88	3.11	1.55	3.96	3.64		
		Week <sub>2</sub>	4.18	4.12	7.33	6.4	8.11	4.78	5.65	3.79	5.74	6.11		
	P/Harcourt	Week <sub>4</sub>	13.11	5.11	14.5	9.32	13.6	8.39	8.39	8.7	8.94	9.31		
		Week <sub>6</sub>	15.23	8.39	16.7	14.74	15.33	9.4	12.33	12.54	14.6	10.73		
		Week <sub>0</sub>	4.02	1.23	2.44	4.05	3.64	4.42	1.8	4.05	3.34	1.55		
		Week <sub>2</sub>	5.91	4.43	5.55	5.11	9.31	13.34	7.83	8.69	5.65	4.23		
		Week <sub>4</sub>	10.42	13.11	12.62	10.65	14.08	16.65	15.45	14.19	15.45	7.82		
		Week <sub>6</sub>	17.18	15.68	18.43	15.31	17.19	18.12	16.46	17.11	17.34	15.56		
	Number of leaf	Lagos	Week <sub>0</sub>	5	4	6	5	4	7	4	4	6	5	
			Week <sub>2</sub>	16	12	13	11	12	11	13	14	12	16	
			Week <sub>4</sub>	26	16	25	20	20	19	23	18	14	21	
			Week <sub>6</sub>	32	33	31	25	38	36	28	28	28	47	
			Week <sub>0</sub>	4	4	3	6	5	4	5	6	6	4	
			Week <sub>2</sub>	20	14	11	12	23	14	15	17	17	17	
P/Harcourt		Week <sub>4</sub>	31	23	24	25	34	26	22	27	33	34		
		Week <sub>6</sub>	59	33	61	45	47	44	36	50	46	56		
		Week <sub>0</sub>	5	5	5	4	4	5	6	6	5	5		
		Week <sub>2</sub>	25	27	19	16	19	17	19	17	19	20		
		Week <sub>4</sub>	39	42	32	28	37	33	39	38	45	37		
		Week <sub>6</sub>	58	77	54	61	51	56	77	57	76	99		
Kano		Week <sub>0</sub>	5	5	6	6	6	3	7	5	4	3		
		Week <sub>2</sub>	20	23	27	24	25	17	23	26	32	22		
		Week <sub>4</sub>	37	47	57	38	45	33	44	42	48	55		
		Week <sub>6</sub>	99	96	74	65	80	79	83	86	66	67		
		Week <sub>0</sub>	11.503	21.221	13.396	21.98	18.759							
		Week <sub>2</sub>	27.331	24.561	28.496	19.856	31.143							
Chlorophyll	Lagos	TOTAL	38.82	45.767	41.877	41.823	49.884							
		A	26.965	27.136	25.617	23.969	15.743							
		B	31.624	34.716	22.612	37.263	40.996							
	P/Harcourt	TOTAL	58.57	61.832	48.215	61.211	56.717							
		A	23.073	24.749	28.51	27.563	25.417							
		B	41.691	37.348	33.158	40.496	34.561							
	Kano	TOTAL	64.74	62.076	61.648	68.036	59.958							
		A	26.225	27.153	32.735	18.536	27.557							
		B	46.817	43.743	31.732	54.864	45.822							
	Kano	TOTAL	73.016	70.912	64.448	73.372								
		Week <sub>0</sub>	0.37	0.44	0.3	0.51	0.33	0.27	0.27	0.25	0.14	0.45		
		Week <sub>2</sub>	0.48	0.49	0.56	0.46	0.63	0.69	0.65	0.44	0.5	0.42		
	Relative growth	P/Harcourt	Week <sub>6</sub>	Kano	0.75	0.8	0.81	0.56	0.73	0.32	0.82	0.7	0.7	0.89
				Gombe	1	1.14	1.03	0.76	1.14	1.14	0.77	0.96	1.04	0.76

Appendix 6: Anatomical study of leaves



Appendix 7: Extraction of Chlorophyll



## Appendix 8: Experimental setup

