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Growth and Leaf Chlorophyll Content of *Eucalyptus grandis* W. Hill ex Maiden are Adversely Affected by Simulated Acid Rain

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Authors' contributions

This work was carried out with collaboration among all authors. Author TFA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LFB and TFA managed the analyses of the study. Author ZNF contributed in editing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study assessed the effects of different pH values of simulated acid rain on growth and leaf chlorophyll content of *Eucalyptus grandis*.

The treatments comprised of two simulated acid rain solutions of pH 4.0 and 3.5, and unacidified water at pH 6.5.

Place and Duration of Study was the National Forestry Development Agency, Humid Savannah Zone, Bamenda I Sub-Division, North West Region, Cameroon, between June and September 2019, respectively.

The experiment was conducted under field conditions. Thirty 3-month-old seedlings were exposed to each pH level at 7-day intervals using a spray bottle. While two sets of thirty seedlings were administered sulphuric acid calibrated tap water with pH values of 4.0 and 3.5, the third set that

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constituted the control was sprayed with normal tap water. Data were collected on morphology, biomass, and leaf chlorophyll content at the end of the study and subjected to analysis of variance and Scheffé's test.

Results: The pH 3.5 treatment resulted in significantly lower responses of height (12.95 cm), number of leaves (15.28), leaf area (23.96 cm^2) and total biomass (0.61 g) than the other two pH levels that did not differ for any of the traits. Average values between pH 4.0 and control were 20.39 cm, 22.00, 45.66 cm^2 and 1.77 g. Stem diameter and root collar diameter declined from 2.87 mm and 4.14 mm at the control to 2.54 mm and 3.78 mm at pH 3.5, respectively. Leaves at pH 3.5 showed signs of necrosis, drying and curling. Leaf chlorophyll content was significantly greater in the control (45.30 SPAD units) than in the pH 4.0 (40.01 SPAD units) and pH 3.5 (39.82 SPAD units) treatments that displayed similar responses.

Conclusion: The study reveals that simulated acid rain at pH 3.5 can have a harmful effect on chlorophyll content and growth of *Eucalyptus grandis*.

Keywords: Broadleaf tree; biomass; morphology; total chlorophyll; acid deposition.

1. INTRODUCTION

Increasing acidity of rain water has been highlighted to be a serious environmental problem affecting each and every component of ecosystem [1]. It is caused by the interaction of sulphur dioxide (SO_2) and nitrogen oxides (NO_x) with water (H_2O) molecules in the atmosphere to form sulphuric and nitric acids that become part of rain reaching the earth's surface. Composite equations of the reactions are as follows:

$$2SO_2\ (g) + O_2\ (g) + 2H_2O\ (l) \rightarrow 2H_2SO_4\ (aq)\ \text{(sulphuric acid)} \quad (1)$$

$$4NO_2\ (g) + O_2\ (g) + 2H_2O\ (l) \rightarrow 4HNO_3\ (aq)\ \text{(nitric acid)} \quad (2)$$

The oxides are emitted from the burning of fossil fuels and volcanoes. Additionally, sulphur dioxide is released from smelting of mineral ores while nitrogen oxides are generated by lightning strikes, wildfires, and agricultural fertilization [2]. Since the pH scale is logarithmic, a decline in one pH unit represents a ten-fold increase in acidity over the higher pH value [3]. The pH of natural, unpolluted, rain is about 5.6 because of the reaction of atmospheric carbon dioxide (CO_2) with water to form weak carbonic acid [4].

$$CO_2\ (g) + H2O\ (l) \rightarrow H_2CO_3\ (aq)\ \text{(carbonic acid)} \quad (3)$$

Thus, rain water that has a pH < 5.6 and [H+] > 2.5 μeq^{-1} is considered to be acidic [5]. However, typical pH values of acid rain are in the range of 3.5 - 5 [6].

Acid rain affects the structure and function of forest plants and plant communities. The effect may be direct and/or indirect. There is evidence that tree growth and reproduction are directly limited by acid rain. According to Lal [7], the decrease in growth occurs when the acidity is from either sulfuric acid alone or when it is combined with nitric acid. While declines in biomass are ascribed to a decrease in photosynthetic capacity [8], the interference with reproduction is likely caused by suppression of growth of certain reproductive structures [3]. For instance, pollen viability and germination have been found to decline with a decrease in pH of simulated acid rain in apple [9] and litchi [10].

Besides, exposure to acidic rain water can result in foliar injury [11], destruction of stomata [12], erosion of epicuticular wax [13], rupture of epidermis [14], an increase in amount of phenolic compounds in mesophyll cells [15], necrosis, chlorosis and folding of leaves [16], crown dieback and whole plant death [17]. Abnormalities in metabolism and the ultrastructure of chloroplasts and mitochondria have also been reported [18]. On the other hand, the growth of plants in low-pH soils may be retarded by low nutrient stress due to root damage [19], suppressed activity or death of organic matter decomposing micro-organisms [13], and leaching of nutrients [20].

Native to the east coast of Australia, *Eucalyptus grandis* W. Hill ex Maiden (family Myrtaceae) has become an important plantation tree in the tropics and subtropics. It is grown from sea level up to 2500 m altitude, in areas with 14-26°C mean annual temperature and 700-4000 mm average annual rainfall [21]. *Eucalyptus grandis* performs best in deep, well drained, fertile loam or clay-loam soils but it is tolerant to impoverished and marginal soils [22]. Significant plantings have been made in Cameroon, especially in the Bamenda Highlands of the North West Region, where the wood is used for general construction, joinery, electricity poles, furniture, and plywood. The tree is also a source of fuelwood and charcoal. Economic returns from the sale of this eucalypt and its products have been quite encouraging. A chemical analysis of 44 monthly rainfall events revealed that rain water in the Bamenda Highlands is acidic (pH 5 in 2012) as is also the case in other parts of the country like the equatorial forested ecosystem area of Zoétélé in the South Region where a slightly lower pH value has been recorded [23,24]. With a scarcity of strong institutions for the regulation of environmental pollution in the developing world [25] and the prospect and quest of rapid industrial development, there will likely be greater emissions of acid oxides leading to rain of even greater acidity including in the Bamenda Highlands region. The incidence and severity of damage to forest plants by acid rain depends on plant species, age of plant and tissue, and environmental conditions among other factors [26]. In this study, the effect of simulated acid rain on growth and leaf chlorophyll content of *Eucalyptus grandis* seedlings was investigated. We tested the hypothesis that a decrease in pH would lead to a reduction in morphological traits, biomass accumulation and chlorophyll content.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted in the nursery of the National Forestry Development Agency, Bamenda I Sub-Division, North West Region, Cameroon. At an altitude of 1250 m, Bamenda is situated between latitude 5.9586 and longitude 10.1475. The municipality is characterized by a rainy season that extends from April to October and a dry season from November to March. Mean annual temperature and rainfall are 21.5°C and 2145 mm [27]. The temperature (mean/min-max) of the months of June, July, and August, September 2019 when the experiment was carried out was 21/16-23, 20/16-23, 20/16-23, and 20/16-22°C and the average monthly rainfall was 752.8, 893.7, 754.8, and 1151 mm, respectively [28].

2.2 Plant Material

The research made use of *Eucalyptus grandis* seedlings that had been raised previously in polythene bags in the National Forestry Agency nursery. Each of the bags had a seedling growing in sandy-loam soil. The plantlets were three months old and of uniform size at the start of the experiment.

2.3 Preparation of Acid Rain Solution

Simulated acid rain solutions were made with sulphuric acid. Tap water was determined to be at pH 5.6 with the aid of a digital pH meter and taken to be the control. The solutions of pH 4 and 3.5 were prepared through a drop-wise addition of concentrated sulphuric acid to tap water amidst verification with the pH meter.

2.4 Experimental Design and Management

Thirty seedlings were exposed to each pH level at 7-day intervals. The treatments were administered using a spray bottle with a plastic nozzle. While two sets of thirty seedlings were sprayed with the corresponding pH corrected water of pH 4 and pH 3.5, the third set that constituted the control was sprayed with normal tap water. During each spraying session, a seedling received 10 ml of solution. Each treatment was replicated twice, making a total of 180 seedlings for the trial. The plants were fertilized with N/P/K 20/10/10 fortnightly and irrigated as necessary. The seedlings were sheltered from rain with an overhead transparent plastic sheet which was mounted on a 2 m tall wooden frame. The treatments were commenced on 14 June and terminated on 7 September 2019.

2.5 Data Collection

At the end of the experiment, nine seedlings were randomly selected from each treatment and replication for data collection. The leaves per plant were counted and the total chlorophyll content of the fifth leaf (counted from the apex) was measured with a chlorophyll meter (SPAD-502, Minolta, Japan). The height, stem diameter, and root collar diameter of the seedling were measured after which the fifth leaf was harvested and its area determined as the number of 1 cm^2 and 0.04 cm^2 cells that fell within the boundary of a traced region of the leaf. The root system of each of the seedlings was rinsed free of substrate and the total biomass recorded after oven drying to constant weight.

2.6 Data Analysis

The null hypothesis was verified by subjecting the data to ANOVA after having ascertained that they fulfilled the assumptions for the test. Means separation was conducted with Scheffé's test. All the statistical tests were performed in Data desk 6.01 at *P* = 0.1.

3. RESULTS

There was a significant effect of treatments on all the growth parameters (Table 1). The values of each trait were lowest at pH 3.5. Differences between the control and pH 4 in height, number of leaves, leaf area, and biomass were not statistically significant (Fig. 1).

On the other hand, the pH 4 treatment did not differ with either the control (pH 5.6) of pH 3.5 for stem and root collar diameter (Fig. 1).

For leaf chlorophyll content, the effect of treatments was marginally significant (Table 1) with values declining from the control to the pH corrected treatments which did not show a significant difference between each other (Table 2). None of the parameters was responsive to either replication or its interaction with pH (Table 1).

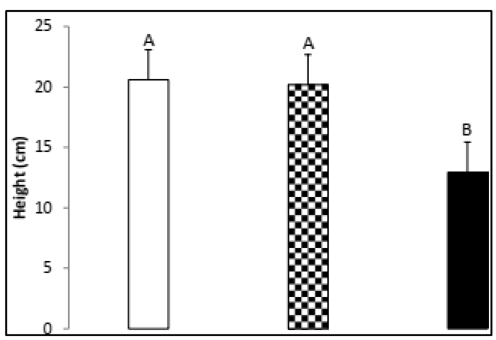
4. DISCUSSION

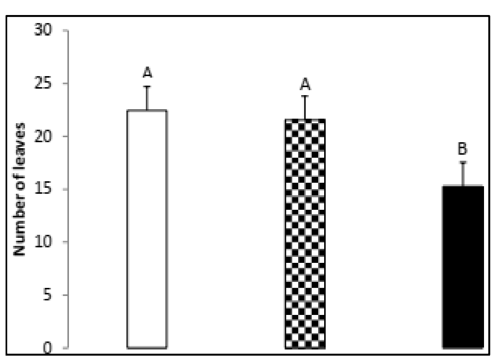
In the present study, values of all the growth parameters decreased with increasing acidity of the simulated acid rain. The most remarkable reductions were observed at the lowest pH level. The data corroborate the findings of other researchers on several other hardwood species. In an investigation of the effect of two simulated acid rain treatments (pH 5.6 versus pH 2.5) and mode of exposure on growth and physiological characteristics of *Elaeocarpus glabripetalus*, for instance, the higher pH treatment level showed superiority in total biomass production, height, and diameter increment [29].

Table 1. ANOVA for the effect of acid rain on growth and chlorophyll content

Source	df	H	SD	RCD	NL	LA	TB	Chl
pH	2	0.0285*	0.0421*	0.0643^{+}	0.0714^{+}	0.0503*	0.0714^{+}	0.1190^{+}
Rep	1	0.5742	0.3864	0.9023	0.2402	0.3244	0.5672	0.7548
pH × Rep	2	0.5798	0.5238	0.5352	0.1579	0.6538	0.6410	0.4626
Error	48							
Total	53							

**, $^{+}$ Significant at P = 0.05 and P = 0.1, respectively*
H = Height, SD = Stem diameter, RCD = Root collar diameter, NL = Number of leaves, LA = Leaf area, TB = Total biomass, Chl = Chlorophyll content, df = Degrees of freedom, Rep = Replication





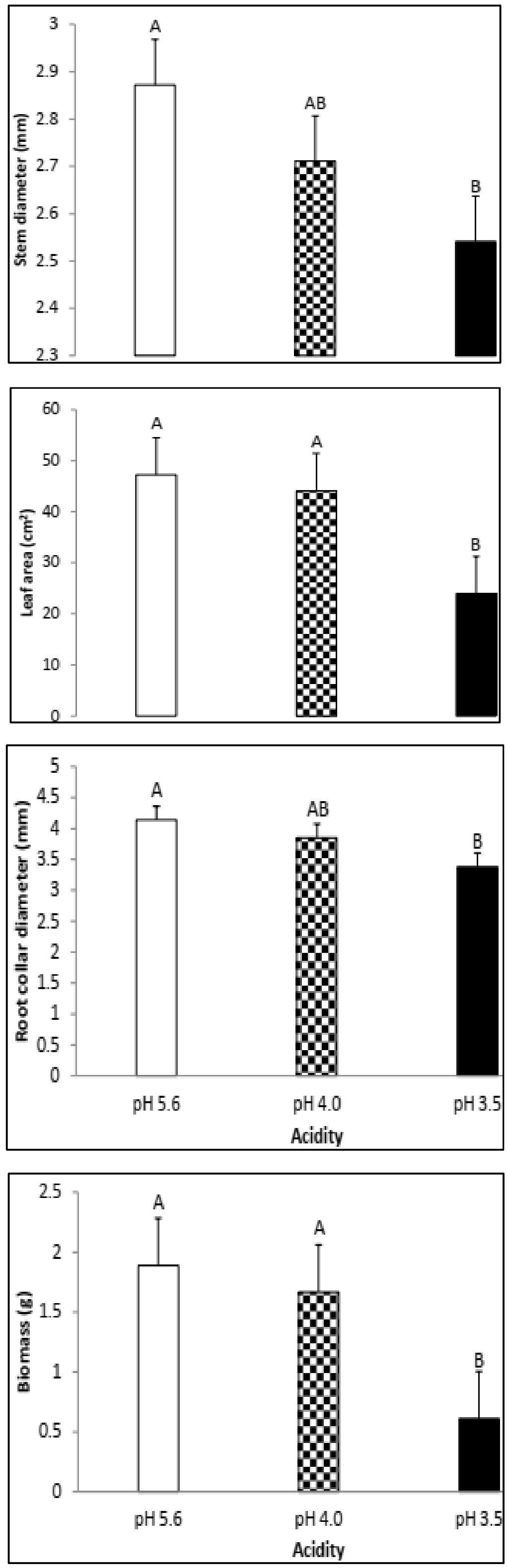


Fig. 1. Effect of acid rain on growth

[AB]Values are means ± SE. Means with underneath different letters are significantly different from one another

Table 2. Effect of acid rain on chlorophyll content

Parameter	Statistic	Acidity		
		pH 5.6	pH 4.0	pH 3.5
Chlorophyll content (SPAD Units)	Mean	45.30^A	40.01^B	39.82^B
	Standard Error	1.62	1.57	1.87
	Range	36.2 - 56.7	35.3 - 50.1	33.0 - 46.3

^{AB}*Means with different superscripts in the row are significantly different at P = 0.1*

The responses of the seedlings were independent of whether the simulant was applied only to the aboveground plant parts, the soil or both the seedling and soil. In *Acacia nilotica*, increasing acid strength of simulated acid rain (pH 3.5, 4.5, 5.5, 6.5, 7.0) in both black and red soil resulted in a decrease in height, root length, number of leaves, total and components biomass, and leaf area among other seedling growth parameters [30]. The outcome of yet another study on the response of seeds and seedlings of five broad-leaved species (*Cinnamomum camphora* L., *Castanopsis fissa*, *Ligustrum lucidum*, *Melia azedarach* L. and *Koelreuteria bipinnata*) to simulated acid rain solutions of pH 2.0, 3.5, 6.0 or to distilled water was that germination in three and growth of all the species are markedly retarded by pH 2.0 [11,31].

The effect of acid rain on higher plants usually arises either via foliage or roots [32]. Simulated acid rain caused damage to the leaves of the *Eucalyptus grandis* seedlings at the lowest pH treatment level. The initial symptom was the formation of necrotic spots which eventually extended to other parts of the leave surface. Some of the leaves curled as the necrotic lesions presented signs of drying and eventually dropped.

Among plant metabolites, pigments are particularly very sensitive to air pollutants and, as such, have been highlighted to be indicators of the physiological state of a plant stressed by acid rain [33]. According to Morrison [34], a decrease in chlorophyll formation in acid rain is due to foliar leaching of nutrient elements including magnesium which is a major component of chlorophyll. In other words, the result of the removal of Mg^{2+} from the tetrapyrol ring of the chlorophyll molecule by the H^+ in acid rain water is the degradation of the pigment [35]. Such was the case with the *Eucalyptus grandis* seedlings in this study where leaf chlorophyll content was significantly higher in the control than the pH adjusted treatments. The declines in chlorophyll content, leaf surface area for light attenuation and the damage to leaves likely contributed to the growth retardation in the acid rain simulated treatments by reducing net photosynthetic rate. Excessive amounts of H^+ have also been shown to adversely affect biological membranes and the photosynthetic electron transport system [7]. Foliar contents of carotenoids were also found to be curtailed by simulated acid rain by other investigators [36].

As mentioned earlier, undesirable effects of simulated acid rain on growth and function of plants may come from belowground [13,19,20]. Although the root system and rhizospheric processes were not examined here, their potential contribution to the overall growth suppression cannot be eliminated because the soil was not protected from the acid simulants during application.

5. CONCLUSION

The findings presented here are in support of this study's prediction of a reduction in growth and chlorophyll content by simulated acid rain. It is, therefore, concluded that simulated acid rain at pH 3.5 can be harmful to chlorophyll content and growth of *Eucalyptus grandis*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bhargava S, Bhargava S. Ecological consequences of the acid rain. IOSR Journal of Applied Chemistry. 2013;5(4): 19-24.
2. Thurston GD. Outdoor air pollution: Sources, atmospheric transport, and human health effects. International Encyclopedia of Public Health. Academic Press. 2008;700-712.
3. Ferenbaugh RW. Acid rain: Biological effects and implications. Boston College

Environmental Affairs Law Review. 1975;4(4):745-756.

4. Dowdey S. How acid rain works; 2007. Available:HowStuffWorks.com, https://science.howstuffworks.com/nature/climate-weather/atmospheric/acid-rain.htm (Accessed 22 January 2020)
5. Seinfeld JH, Pandis SN. Atmospheric chemistry and physics – from air pollution to climate change. New York: John Wiley and Sons, Inc; 1998.
6. Kim MG, Kim OJ, Lee HY. A case study on acid rain over Jeju Island, Korea. Journal of Climate Research. 2007;2(1):33-49.
7. Lal N. Effects of rain on plant growth and development. e-Journal of Science and Technology. 2016;11(5):85-108.
8. Neufeld HS, Jernstedt JA, Haines BL. Direct foliar effects of simulated acid rain. I. Damage, growth and gas exchange. The New Phytologist. 1985;99(3):389-405.
9. Bellani LM, Rinallo C, Muccifora S, Gori P. Effects of simulated acid rain on pollen physiology and ultrastructure in the apple. Environ. Pollution. 1997;95(3):357-362.
10. Qiu DL, Liu XH, Guo SZ. Effects of simulated acid rain on fertility of litchi. Journal of Environmental Science. 2005; 17(6):1034-1037.
11. Fan HB, Wang YH. Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seedling growth of five hardwood species growing in China. Forest Ecology and Management. 2000;126(3):321-329.
12. Sant'Anna-Santos BF, Campos da Silva L, Azevedo AA, Aguiar R. Effects of simulated acid rain on leaf anatomy and micromorphology of *Genipa americana* L. (Rubiaceae). Brazilian Archives of Biology and Technology. 2006;49(2):313-321.
13. Mohajan HK. Acid rain is local environment pollution but global concern. Open Science Journal of Analytical Chemistry. 2018;3(5): 47-55.
14. Sant' Anna-Santos BF. Campos da Silva L, Azevedo AA, Marcos de Araujo J, Alves EF, Monteiro da Silva EA, Rosane Aguiar R. Effects of simulated acid rain on the foliar micromorphology and anatomy of tree tropical species. Environmental and Experimental Botany. 2006;58(1-3):158-168.
15. Zobel A, Nighswander JE. Accumulation of phenolic compounds in the necrotic areas of Austrian and red pine needles after spraying with sulphuric acid: A possible bioindicator of air pollution. The New Phytologist. 1991;117(4):565-574.
16. Odiyi BO, Eniola AO. The effect of simulated acid rain on plant growth component of cowpea (*Vigna unguiculata*) L. Walps. Jordan Journal of Biological Sciences. 2015;8(1):51-54.
17. Tomilnson G. Air pollutants and forest decline. Environmental Science and Technology. 1983;17(6):246-256.
18. Gabara B, Sklodowska M, Wyrwicka A, Glinska S, Gapinska M. Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. Plant Science. 2003;164(4):507-516.
19. Ulrich B, Mayer R, Khanna PK. Chemical changes due to acid precipitation in loess-derived soil in Central Europe. Soil Science. 1980;130(4):193-199.
20. Nawaz R, Parkpian P, Garivait H, Anurakpongsatorn P, DeLaune RD, Jugsujinda A. Impacts of acid rain on base cations, aluminium and acidity development in highly weathered soils of Thailand. Communications in Soil Science and Plant Analysis. 2012;43(10):1382-1400.
21. Nyunaï N. *Eucalyptus grandis* W. Hill ex Maiden. In: Louppe D, Oteng-Amoako AA, Brink M. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands; 2008. (Accessed 23 January 2020)
22. Ssenku JE, Ntale M, Backéus E, Oryem-Origa H. Assessment of seedling establishment and growth performance of *Leucaena leucocephala* (Lam.) De Wit., *Senna siamea* (Lam.) and *Eucalyptus grandis* W. Hill ex Maid. in amended and untreated pyrite and copper tailings. Journal of Biosciences and Medicine. 2014;2:33-50.
23. Sigha-Nkamdjou L, Galy-Lacaux C, Pont V, Richard S, Sighomnou D, Lacaux JP. Rainwater chemistry and wet deposition over the equatorial forested ecosystem of Zoétélé (Cameroon). Journal of Atmospheric Chemistry. 2003;46:173-198.
24. Wirmvem MJ, Ohba T, Fantong WY, Ayonghe SN, Hogarh JN, Suila JY, Asaah AN, Ooki S, Tanyileke G, Hell JV. Origin of major ions in monthly rainfall events at the Bamenda Highlands, North West

Cameroon. Journal of Environmental Sciences. 2014;26(4):801-809.
25. Bell RG, Russel C. Environmental policy for developing countries. Issues in Science and Technology. 2002;18(3).
26. Dickison WC. Integrative plant anatomy. Massachusetts: Harcourt/ Academic Press; 2000.
27. Climate-Data.org. Bamenda climate: Bamenda climate summary. Available:https://en.climate-data.org/africa/cameroon/northwest/bamenda-2905/ (Accessed 03 February 2020)
28. World Weather Online. Bamenda monthly climate averages. Available:https://www.worldweatheronline.com/bamenda-weather-averages/nord-ouest/cm.aspx (Accessed 30 January 2020)
29. Yu F, Song Q, Liu M, Dong L, Yi L. Effects of different acid rain treatments on biomass allocation and physiological characteristics in *Elaeocarpus glabripetalus* seedlings. Scientia Silvae Sinicae. 2016;52(5):92-100.
30. Balasubramanian G, Udayasoorian C, Prabu PC. Effects of short-term exposure of simulated acid rain on the growth of *Acacia nilotica*. Journal of Tropical Forest Science. 2007;19(4):198-206.
31. Fan HB, Li C. Effects of simulated acid rain on seedlings emergence and growth of five broad-leaved species. Journal of Forestry Research. 1999;10(2):83-86.
32. Mallick B, Behera S, Tiwari TN, Mishra PC. Risk of acid attack on plants: A review. Proceedings of the International Conference on Energy, Environment and Material Science. Agios Nikolaos, Crete, Greece; 2015.
33. Sensor M, Kloos M, Luto C. Influence of soil substrate and ozone plus acid mist on the pigment content and composition of needles from young Norway spruce trees. Environmental Pollution. 1990;64(3-4):295-314.
34. Morrison IK. Acid rain, forests and forestry. In: Stone, E.L. (Ed.), Proceedings of the 6th North American Forest Soils Conference, University of Tennessee, Knoxville. 1984;209-219.
35. Foster JR. Influence of pH and plant nutrients status onion fluxes between tomato plants and simulated acid mists. New Phytologist. 1990;116(3):475-485.
36. Khan AA, Mustabeen. Observation of simulated acid rain impact on chickpea plant. ECOPRINT. 2013;20:77-80.

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