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Stress Induced by Simulated Nitric and Sulphuric Acid Rain on the Nutrient Quality of *Cucurbita Moschata* (Duchesne Ex Poir)

By A. A. J. Mofunanya
University of Calaba

Abstract- Stress has been associated with several alterations in the physiological and biochemical composition of plants affecting their use in chemotherapy. Investigations were carried out to assess simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) stress on the medicinal quality of Cucurbita moschata. Results revealed that all the nutrients investigated had higher values at pH 6.0 than at pH 2.0, 3.0 and 4.0. The presence or absence of phytochemicals was not affected by SNAR and SSAR levels. Stress induced on ash, fat, fibre, carbohydrate and moisture caused significant (P=0.05) increase at pH 2.0 and 3.0 with decrease at pH 4.0 with the exception of leaf protein which showed increase at all levels of acidity compared to the control pH 6.0. Amino acids depicted a trend similar to that of protein. Potassium, sodium, calcium, magnesium, iron, copper, zinc, manganese and P were seriously affected by simulated acid rain stress. Stress caused an increase in vitamin A and the B-complex vitamins at pH concentration 2.0 with a decrease at concentration 3.0 and 4.0. While vitamin C was significantly reduced by simulated acid rain stress, vitamin K showed increase in content at all levels of acidity.

Keywords: *cucurbita moschata, medicinal quality, stress, simulated nitric and sulphuric acid rain.*

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I. Introduction

Plants are constantly being exposed to both biotic and abiotic stress in their growing environment. Stress is induced by factors in the plant environment. Plant stress is defined as 'any unsuitable condition or substance that alters or hinders the growth and development of the plant'. Larcher referred to stress as 'changes in physiology' that occurs when species are exposed to unusual conditions that need not pose a threat to life but will cause an alarm response (Kranner *et al*., 2010). Abiotic stress is basically unavoidable and directly affects not only plant growth and productivity but also the chemical composition of crop plants. Prolong exposure of plants to acid rain is accompanied by oxidative stress as it is with other stressors in the plant environment. The negative effect of stress is often mediated by the oxidative damage initiated by reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Gill and Tuteja, 2010). Reactive oxygen species (ROS) production during environmental stress is one of the major causes for decreases in crop productivity, injury and death. Any situation in which cellular redox homoeostasis is impeded can lead to oxidative stress or reactive ROS generation (Asada, 1994). Stress is evident by the production of ROS.

Environmental stress is directly linked to increased accumulation of ROS. ROS production are increased by several environmental factors of stress, such as exposition to high levels of light, drought, heavy metals, salt concentrations, temperature extremes, air pollution, high irradiation (UV radiation), herbicides and pathogen attacks. Whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratão *et al*., 2005). The balance between production and removal of ROS may be altered by a number of biotic and abiotic factors, which may increase the intercellular levels of ROS (Apel and Hirt, 2004). When the level of ROS increase exceeds the cell defense mechanisms, the cell is in a state of oxidative stress (Sharma *et al*., 2012).

In plants, ROS act as secondary messengers in various key physiological processes and they induce oxidative damages under several environmental stress conditions when the balance between ROS production and elimination, necessary for normal cellular homeostasis, is disturbed. Reactive oxygen species are highly reactive and toxic by-product of aerobic pathways of metabolism, causing oxidative damages evidenced in the form of degradation of biomolecules such as pigments, proteins, nucleic acids, lipids, carbohydrates, and DNA, which result in plant cellular death (Gill and Tuteja, 2010; Kapoor *et al*., 2015; Singh *et al*., 2016). ROS play an important role of signaling in plants thus, controlling processes such as growth, development and response to biotic and abiotic environmental stimuli. The formation of reactive oxygen species has been described as being a result of several abiotic stresses, such as simulated acid rain Velikova *et al*. (2000), the application of herbicides (Song *et al*., 2007), pathogens (Mofunanya, 2015). Hippeli and Elstner (1996) observed that stress induced by acid rain components; SO_2 and NO_X resulted in the generation of reactive free oxygen radicals that causes inhibition of photosynthesis,

Author: Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria. e-mail: amofunanya@yahoo.com

enzyme degradation, damage to membrane, alterations in DNA, all leading to reduction in growth. Also observed was an increase in H_2O_2 level and MDA accumulation, an indication that oxidative stress has been induced in connection to membrane damage after a single spray of acid rain on bean plant (Velikova *et al*., 2000). Velikova *et al*. (2002) reported that stress induced by simulated acid rain treatment of pH 1.8 on *Phaseolus vulgaris* caused increase in H_2O_2 and MDA (malonyldialdehyde) which are end product of lipid peroxidation.

Cucurbita moschata (Duchesne ex Poir) is a member of the family Cucurbitaceae. Members of the family Cucurbitaceae are commonly called cucurbits which constitute a very large group with approproximately130 genera and 800 species. *Cucurbita moschata* is a squash native to Central America, northern and southern America. It is widely cultivated for its edible leaves and fruits in warm temperate and tropical areas. *Cucurbita moschata* is an indigenous vegetable in Nigeria. For a long time it has been an essential gradient in the diet of the rural communities as well as urban people in Nigeria. The leaves of *C. moschata* are sold in Nigerian markets as a vegetable and fruits used in various delicacies. It is a seasonal crop used traditionally as food for both human and animal (Doymaz, 2007). The young leaves and stems, flowers, young and ripe fruits of *C. moschata* are eaten as vegetable. The latter are also commonly used to prepare sweets and as fodder. The seeds are eaten whole, roasted or toasted and are ground into powder and used in different stews (Bates *et al*., 1990).

Nigeria is a country with a population of 198,000.000 people (NPC, 2018). Owing to this population increase, there is a corresponding rise in anthropogenic (human) activities which have given rise to acid rain precursor gases. Nigeria is the highest importer of used vehicles, with no guarantee on combustion efficiency. In accordance to the April 2006 edition of African Review of Business and Technology, London-based magazine survey, owing to power outages, Nigeria is also the largest importer of both petrol and diesel generators, estimated to the cost of $152 million per annum, with their attendant environmental and health implications. The acid rain precursors (SO_x and NO_x) that cause acid rain are transboundary pollutants. There are the same acid rain precursor gases anywhere in the World. In rural and urban cities in Nigeria, worn out tires are used to roast cow skin or hide, goat and poultry in abattoirs, which releases high quantities of SO_2 into the atmosphere. Oxides of nitrogen are (NO_x) are by-products of firing processes of extremely high temperature such as automobiles, utility plants and in chemical industries like fertilizer producing industries (Nduka *et al*., 2008). Due to these anthropogenic activities, cases of acidic rain are abound in Nigeria. Plants which are the primary producers are severely affected by acid rain.

Stress has been associated with several alterations in the physiological and biochemical composition of plants affecting their use in chemotherapy. Plant nutrients have been implicated in disease management. Plants have been in use for human welfare over the millennia in health promotion, drugs and for fragrance. The greater portions of the people in the rural areas of developing countries use plant-based traditional medicines for health care. *Cucurbita moschata* is a valued vegetable crop eaten by Nigerians. Considerable levels of acid rains have been determined in different parts of Nigeria on growth and yield of crop plants. Mofunanya and Egah (2017) studied the effect of simulated nitric and sulphuric acid rains on the nutrient content of *Amaranthus hybridus* in Nigeria. However, no literature exists in Nigeria on simulated acid rain effect on the nutrient quality of *C. moschata*. The present study seek to investigate stress induced individually by simulated nitric acid rain (SNAR) and simulated sulphuric acid rain (SSAR) on the medicinal quality of *Cucurbita moschata* leaf, stem and root.

II. Seeds and Materials Collection

Seeds of *Cucurbita moschata* were provided by a farmer in Akparabong, Ikom Local Government Area of Cross River State, Nigeria. Polyethylene bags of 16 mm in diameter were bought from the Ministry of Agriculture, Calabar. Nitric and sulphuric acids were purchased from a Scientific Shop all in Calabar, Nigeria. The Seeds were planted in poly bags, two seeds per bag. On germination, the seedlings were allowed to stay for a period of two weeks with regular application of distilled water. Simulated nutric and sulphuric acid rain application began after two weeks of germination. Planting and growth of *C. moschata* was carried out in the Department of Botany greenhouse, University of Calabar, Calabar, Nigeria (latitude 4.952'N and longitude 8.341'E) at 25±3°C to minimize pest infestation and uniformity of sunlight and water supply.

III. Simulated acid Rain Preparation and Application

Simulated nitric and sulphuric acid rain (SNAR and SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately prepared and a control pH of 6.0. Each pH concentration (HNO_3 or H_2SO_4) was prepared using different amount of acid. Thirty milliliters (30 ml) of each acid was used for pH concentrations of 2.0, 20.1 ml for pH 3.0 and 10.2 ml for pH 4.0 using a Deluxe pH meter and distilled water. Distilled water of pH 6.0 was used as control. A total of thirty five poly bags were used; fifteen bags for simulated nitric acid rain, fifteen bags for simulated sulphuric acid rain and five bags for the control that is, five bags for each pH concentration replicated three times were used in this study. Prior to

acid rain application, the poly bags were arranged in a completely randomized block design. Fifty milliliters (50 ml) of simulated acid rain concentration was applied at the initial growth period, this amount however, was increase with increase in growth. Spraying of simulated acid rain concentrations was done using a domestic hand-spraying unit on the plants as well as soil. Application was done at an interval of two days for thirteen weeks.

IV. Sample Preparation

At the end of thirteen weeks post simulated acid rain application, the whole plants of *C. moshata* grown at various pH concentrations were harvested and the plant parts (Leaf, Stem and Root) separated. The roots were washed in tape water to remove soil before sun-drying along with other plant parts for one week. The different parts were milled separately into powder in an electric mill (National Food Grinder, Model MK 308, Japan).The pulverized samples were used to analyzed the potential of simulated nitric and sulphuric acid rains stress on proximate, amino acids, vitamins and antinutrients contents of *Cucurbita moschata*.

V. Sample Analysis

Standard methods of plant samples analysis were employed. Crude protein was analyzed using the Kjeldahl method. Fat content was determined by AOAC (1995). Ash, fibre, carbohydrate and Vitamin contents of *C. moschata* were analyzed by the method of AOAC (2006). Amino acids content of samples was determined by the method of Speckman (1956; AOAC, 2006).

VI. Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science, Version 15.0 [SPSS, 2003]. Results were also expressed as percentage differences and differences between means were determined at 5% level of probability.

VII. Results

Table 1: Stress induced by simulated nitric and sulphuric acid rain on proximate nutrients of *Cucurbita moschata*

mg/100g								
Proximate nutrients	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Ash	Leaf	4.61± 0.01(18.2)	4.14±0.01(6.2)	3.95± 0.01(1.3)	3.27±0.01(16.2)	3.30 ±0.01(15.4)	3.40± 0.01(12.8)	3.90± 0.01
	Stem	4.70 ± 0.01(24.0)	3.99 ± 0.01(5.3)	3.81 ± 0.01(0.5)	4.84 ± 0.01(1.3)	3.95 ± 0.01(1.3)	3.79± 0.01(0.0)	3.79 ± 0.01
	Root	2.94± 0.01(37.4)	3.60± 0.01(23.4)	4.30± 0.016(8.5)	3.25± 0.01(30.9)	3.45± 0.01(26.6)	4.21± 0.01(10.4)	4.70± 0.01
Protein	Leaf	5.23 ± 0.01(54.7)	4.70±0.01(39.1)	4.10± 0.01(21.3)	5.16± 0.01(52.7)	4.54± 0.01(34.3)	3.78± 0.01(11.8)	3.38± 0.01
	Stem	6.01 ± 0.01(22.7)	4.18 ± 0.01(14.7)	3.84 ± 0.01(21.6)	5.80 ± 0.01(18.4)	5.28 ± 0.01(7.8)	4.14 ± 0.01(15.5)	4.90 ± 0.01
	Root	5.63 ± 0.01(28.5)	4.70± 0.01(7.3)	3.90± 0.01(11.0)	5.60± 0.01(27.9)	4.78± 0.01(9.1)	4.10± 0.01(6.4)	4.38± 0.01
Fat	Leaf	0.52 ± 0.12(36.6)	0.57 ± 0.02(30.5)	0.70 ± 0.15(14.6)	0.50 ± 0.02(39.0)	0.57 ± 0.01(30.5)	0.73 ± 0.01(11.0)	0.82 ± 0.02
	Stem	0.49 ± 0.01(38.8)	0.55 ± 0.01(31.3)	0.64 ± 0.01(20.0)	0. 51 ± 0.01(36.3)	0.62 ± 0.01(10.8)	0.75 ± 0.01(6.3)	0.80 ± 0.01
	Root	0.45 ± 0.01(22.4)	0.48 ± 0.01(17.2)	0.53 ± 0.02(8.6)	0.47 ± 0.02(19.0)	0.50 ± 0.01(13.8)	0.52 ± 0.01(10.3)	0.58 ± 0.01
Fibre	Leaf	5.30±0.01(51.9)	4.68± 0.01(34.1)	4.50 ± 0.01(28.9)	5.50±0.01(57.6)	4.75±0.01(36.1)	4.63±0.01(32.7)	3.49±0.01
	Stem	2.70±0.01(31.5)	2.85± 0.01(27.7)	3.25±0.01(17.5)	3.50±0.01(11.2)	3.54 ± 0.01(10.2)	3.90 ± 0.01(1.0)	3.94 ± 0.01
	Root	3.71± 0.01(28.4)	3.34± 0.01(15.6)	2.65± 0.01(8.3)	3.80± 0.01(31.5)	3.52± 0.01(21.8)	2.70± 0.01(6.6)	2.89± 0.01
Carbohydrate	Leaf	81.06± 0.01(4.2)	81.78 ±0.01(3.3)	81.78 ±0.01(2.6)	82.40± 0.01(2.6)	82.64± 0.01(2.3)	82.80± 0.01(2.1)	84.58± 0.01
	Stem	81.76 ± 0.01(3.3)	82.76 ± 0.01(2.2)	83.67 ± 0.01(1.1)	82.37 ± 0.01(2.6)	82.81± 0.01(2.1)	83.90 ± 0.01(0.8)	84.58 ± 0.01
	Root	85.10± 0.01(2.8)	84.22± 0.01(1.7)	83.90± 0.01(1.3)	84.51± 0.01(2.0)	84.72± 0.01(2.3)	84.80± 0.01(2.4)	82.82± 0.01

- Results are mean ± SE, n = 3 determinations, P=0.05, numbers in parenthesis are percentage difference
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and Simulated acid rain treated sample as a percentage of the control.

a) Stress induced by simulated nitric and sulphuric acid rain on proximate nutrients of Cucurbita moschata

Results analysis showed that proximate nutrients of *Cucurbita moschata* in the control plant parts were either higher or lower than the acid rain treated plant parts. Acid rain stress resulted in significant (P=0.05) increase or decrease in some proximate nutrients. Increase or decrease in proximate nutrients of *C. moschata* was in accordance with simulated acid rain concentration and amount in plant parts. Highest increase or decrease occurred at pH 2.0 and lowest at pH 4.0. Simulated sulphuric acid rain (SSAR) caused a decrease in ash content of leaf and root with an increase in stem content when compared to the control. At all acidity levels protein content of leaf increased significantly. Percentage increase of leaf protein showed values of 54.7%, 39.1%, 21.5% and 52.7%, 34.3%, 11.8%. Stem and root protein content was significantly increased at pH 2.0 and 3.0 with percentage increased values of 22.7%, 14.7% for SNAR and 18.1%, 7.8% for SSAR and 28.5%, 7.3%, SNAR and 27.9%, 9.1% for SSAR. A decrease in stem and root protein at pH 4.0 had percentage values 21.6%, 11.0% and 15.5%, 6.4% for SNAR and SSAR respectively. Decrease in leaf and root fat content with an increase in stem fat content due to acid rain stress was obtained. Simulated nitric acid rain (SNAR) stress led to significant increase in ash content of leaf and stem with decrease in ash content of root. SNAR and SSAR stress caused an increase in leaf fibre content at pH 2.0, 3.0 and 4.0 with percentage increase values of 51.9%, 34.1%, 24.6% and 57.6%, 36.5%, 32.7% respectively. Fibre in stem showed decrease in content at all levels of acidity with percentage decrease in values of 31.5%, 27.7%, 17.5% and 11.2%, 10.2% and 1.0% respectively for SNAR and SSAR. However, root fibre content depicted increase at pH 2.0 and 3.0 percentage increase values of 28.4%, 15.6% and 31.5%, 21.8% with decrease at pH 4.0 of 8.3% and 6.6% for both simulated acid rain. An increase in root fibre content of 28.4%, 15.6% and 31.5%, 21.8% at pH 2.0 and 3.0 and a decrease of 8.3% and 6.6% at pH 4.0 for SNAR and SSAR induced stress. SNAR and SSAR stress induced increase in root carbohydrate content compared to control (Table 1).

Table 2: Stress induced by simulated nitric and sulphuric acid rain on amino acids content of *Cucurbita moschata*

g/16 N								
Amino acid	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Histidine	Leaf	4.60±0.01(36.1)	4.35±001 (28.7)	4.10±0.01 (21.3)	4.45±0.01 (31.7)	4.20± 0.01 (24.3)	3.78±0.01(11.8)	3.38± 0.01
	Stem	5.50 ± 0.01(14.3)	5.29±0.01 (10.0)	4.36± 0.01 (9.4)	5.35 ± 0.01 (11.2)	5.19 ± 0.01 (7.9)	4.29 ± 0.01 (10.8)	4.81 ± 0.01
	Root	4.80± 0.01 (12.1)	4.50± 0.01 (5.1)	3.83± 0.01 (10.5)	4.60± 0.01 (7.5)	4.50± 0.01 (5.1)	3.67± 0.01 (14.3)	4.28± 0.01
Lysine	Leaf	6.66 ± 0.1 (28.8)	5.75±0.06 (10.6)	4.75 ± 0.03 (8.1)	6.18 ± 0.02 (19.5)	5.51 ± 0.01 (6.6)	5.20 ±0.02 (0.6)	5.17 ± 0.1
	Stem	4.70 ± 0.02 (20.2)	4.50±0.02 (15.1)	3.70 ± 0.03 (5.4)	4.52 ± 0.3 (15.6)	4.39 ± 0.01 (12.3)	3.57 ±0.01 (8.7)	3.91 ± 0.02
	Root	3.62 ± 0.03 (28.4)	3.48±0.02 (23.4)	2.68 ± 0.02 (6.0)	3.37 ± 0.02 (19.5)	3.23 ± 0.01 (14.5)	2.60 ± 0.01 (7.8)	2.82 ± 0.02
Arginine	Leaf	7.94 ± 0.01 (13.3)	7.45 ±0.01 (6.3)	7.19 ± 0.1 (2.6)	7.63± 0.01 (8.8)	7.41 ± 0.01 (5.7)	7.22 ± 0.01 (3.0)	7.01 ± 0.01
	Stem	7.02 ± 0.1 (10.2)	6.78 ± 0.01 (6.4)	6.10 ± 0.01 (4.2)	6.92 ± 0.02 (8.6)	6.62 ± 0.01 (3.9)	6.10 ± 0.01 (4.2)	6.37 ± 0.02
	Root	3.47 ± 0.01 (23.5)	3.05 ± 0.1 (8.5)	2.65 ± 0.01 (5.7)	3.35 ± 0.01 (19.2)	3.00 ± 0.01 (6.8)	2.49 ± 0.01 (11.4)	2.81 ± 0.02
Aspartic acid	Leaf	13.70± 0.02 (42.6)	11.55±0.02(20.2)	9.83 ± 0.02 (2.3)	13.47± 0.06 (40.2)	11.40± 0.01 (18.6)	9.75 ± 0.01 (1.5)	9.61 ± 0.06
	Stem	12.02± 0.01 (46.9)	10.64±0.01(30.1)	9.80 ±0.01 (19.8)	12.00± 0.01 (46.7)	10.46± 0.06 (27.9)	9.70 ± 0.01 (18.6)	8.18 ± 0.06
	Root	7.26 ± 0.01 (12.6)	7.03 ± 0.01 (9.0)	6.89 ±0.01 (6.8)	7.20 ± 0.01 (11.6)	6.98 ±0.01 (8.2)	6.75 ± 0.01 (4.7)	6.45 ± 0.03
Threonine	Leaf	4.96 ± 0.01 (16.4)	4.50 ±0.01 (5.6)	4.32 ± 0.01 (1.4)	4.71 ± 0.02 (10.6)	4.60 ± 0.01 (8.0)	4.30 ± 0.01 (0.9)	4.26 ± 0.1
	Stem	3.83 ± 0.02 (19.7)	3.79 ±0.01 (18.4)	3.06 ± 0.01 (4.4)	3.72 ± 0.01 (16.3)	3.40 ± 0.1 (9.4)	3.12 ± 0.01 (2.5)	3.20 ± 0.01
	Root	3.51 ± 0.06 (49.4)	2.86± 0.01 (21.7)	2.11 ± 0.02 (10.2)	3.32 ± 0.01 (41.3)	2.63 ± 0.02 (11.9)	2.20 ± 0.1 (6.4)	2.35 ± 0.01
Serine	Leaf	3.01± 0.01 (15.3)	2.80 ± 0.01 (7.3)	2.71 ± 0.01 (7.3)	3.28 ± 0.01 (25.7)	2.95± 0.1 (13.0)	2.80 ± 0.1 (7.3)	2.61 ± 0.03
	Stem	2.76 ± 0.2 (10.4)	2.60 ± 0.1 (4.0)	2.02 ± 0.01 (19.2)	2.85 ± 0.1 (14.0)	2.69 ± 0.01 (7.6)	1.98 ± 0.01 (20.8)	2.50 ± 0.01
	Root	1.78 ± 0.03 (26.2)	1.50 ± 0.03 (6.4)	1.20 ± 0.1 (14.9)	1.85 ± 0.01 (31.2)	1.55 ± 0.01 (9.9)	1.16 ± 0.1 (17.7)	1.41 ± 0.01
Glutamic acid	Leaf	13.73± 0.02 (12.6)	12.40± 0.01 (9.9)	12.31 ± 0.02 (1.0)	13.71± 0.02 (12.5)	13.24± 0.02 (8.6)	12.21± 0.02 (0.2)	12.19 ± 0.1
	Stem	13.48± 0.02 (10.3)	12.79± 0.01 (6.5)	12.45 ± 0.02 (4.0)	13.42 ± 0.02 (7.7)	12.64 ± 0.02 (5.6)	12.39 ± 0.02 (4.8)	12.10 ± 0.1
	Root	8.65 ± 0.01 (12.8)	8.17 ± 0.01 (6.5)	7.83 ± 0.1 (2.1)	8.45 ± 0.2 (10.2)	8.10 ± 0.02 (5.6)	7.78 ± 0.01(1.4)	7.67 ± 0.2
Proline	Leaf	3.93 ± 0.1 (38.9)	3.67 ± 0.1 (29.7)	2.90 ± 0.1 (2.5)	3.69 ± 0.1 (30.4)	3.46 ± 0.1 (22.3)	2.86 ±0.2 (1.1)	2.83 ± 0.06
	Stem	3.67 ± 0.1 (26.6)	2.95 ± 0.01 (9.7)	2.50 ± 0.1 (7.1)	3.40 ± 0.1 (26.4)	2.85 ± 0.1 (5.9)	2.50 ±0.1 (7.1)	2.69 ± 0.03
	Root	1.95 ± 0.1 (54.5)	1.71 ± 0.1 (34.6)	1.19 ± 0.1 (6.3)	1.80 ± 0.1 (41.7)	1.65 ± 0.1 (29.9)	1.11± 0.01 (7.9)	1.27 ± 0.03
Glysine	Leaf	5.83 ± 0.01 (23.3)	5.27± 0.01 (11.4)	4.88 ± 0.01 (3.2)	5.58 ± 0.01 (18.0)	5.07 ± 0.01 (7.2)	4.83 ± 0.01 (7.2)	4.73 ± 0.02
	Stem	2.95 ± 0.01 (28.3)	2.51 ± 0.01 (9.1)	1.97 ± 0.01 (14.3)	2.95 ± 0.01 (28.3)	2.46 ± 0.01 (7.0)	2.01 ± 0.01 (12.6)	2.30 ± 0.02
	Root	1.60 ± 0.01 (33.3)	1.45±0.01 (20.8)	0.99 ± 0.01 (17.5)	1.60 ±0.01 (33.3)	1.41 ± 0.1 (17.5)	1.05 ± 0.1 (12.5)	1.20 ± 0.02
Alanine	Leaf	4.30 ± 0.01 (22.9)	3.97 ± 0.1 (13.4)	3.62 ± 0.01 (3.4)	4.53 ± 0.01 (29.4)	3.99 ± 0.01 (14.0)	3.60 ± 0.01 (2.9)	3.50 ± 0.1
	Stem	3.79 ± 0.02 (16.3)	3.61 ± 0.1 (10.7)	2.95 ± 0.02 (9.5)	3.71 ± 0.01 (13.8)	3.50 ± 0.01 (7.4)	3.00 ± 0.01 (8.0)	3.26 ± 0.1
	Root	1.57 ± 0.02 (53.7)	1.25± 0.02 (22.5)	0.90 ± 0.02 (12.8)	1.54 ± 0.2 (51.0)	1.15 ± 0.02 (12.7)	0.94 ± 0.02 (7.8)	1.02 ± 0.1

Cysteine	Leaf	0.96 ± 0.1 (14.3)	0.89 ± 0.1 (6.0)	0.86 ± 0.1 (2.4)	0.95 ± 0.1 (13.1)	0.87 ± 0.1 (3.6)	0.86 ± 0.1 (2.4)	0.84 ± 0.01
	Stem	0.90 ± 0.1 (12.2)	0.85 ± 0.1 (6.3)	0.76 ± 0.1 (5.0)	0.90 ± 0.1 (12.2)	0.84 ± 0.1 (5.0)	0.74 ± 0.1 (7.5)	0.80 ± 0.02
	Root	0.60 ± 0.1 (20.0)	0.54 ± 0.1 (8.0)	0.45 ± 0.1 (16.0)	0.57 ± 0.1 (14.0)	0.55 ± 0.1 (8.2)	0.45 ± 0.1 (10.0)	0.50 ± 0.1
Valine	Leaf	4.88 ± 0.2 (12.2)	4.71 ± 0.03 (8.3)	4.72 ± 0.0 (3.9)	4.61± 0.1 (8.5)	4.20 ± 0.1 (6.0)	3.99 ± 0.1 (8.3)	4.35 ± 0.1
	Stem	4.96 ± 0.2 (12.5)	4.70 ± 0.1 (6.6)	3.79 ± 0.06 (14.1)	4.87 ± 0.1 (10.4)	4.65 ± 0.1 (5.4)	3.81 ± 0.1 (13.6)	4.41 ± 0.2
	Root	3.20 ± 0.1 (42.9)	3.03 ± 0.2 (35.3)	1.83 ± 0.2 (18.3)	3.11 ± 0.1 (38.8)	2.78 ± 0.1 24.1()	1.91 ± 0.1 (14.7)	2.24 ± 0.2
Methionine	Leaf	1.65 ± 0.02 (24.1)	1.51± 0.02 (13.5)	1.38 ± 0.02 (3.8)	1.79 ± 0.03 (34.6)	1.62 ± 0.2 (21.8)	1.42 ± 0.2 (6.8)	1.33 ± 0.01
	Stem	1.59 ± 0.02 (19.0)	1.47± 0.01 (16.7)	1.10 ± 0.02 (12.7)	1.65 ± 0.2 (31.0)	1.51± 0.2 (19.8)	1.10 ± 0.2 (12.7)	1.26 ± 0.01
	Root	1.16 ± 0.01 (39.8)	0.97±0.01 (16.9)	0.68 ± 0.01 (18.1)	1.22 ± 0.2 (47.0)	1.02 ± 0.2 (22.9)	0.70 ± 0.1 (15.7)	0.83 ± 0.01
Isoleucine	Leaf	4.71 ± 0.01 (6.3)	4.55 ± 0.3 (2.7)	4.48 ± 0.1 (1.1)	4.82 ± 0.02 (8.8)	4.60 ± 0.01 (3.8)	4.50 ± 0.01 (1.6)	4.43 ± 0.2
	Stem	5.20 ± 0.2 (8.3)	4.95 ± 0.2 (3.1)	4.66 ± 0.1 (2.9)	5.47 ± 0.01 (6.9)	5.03 ± 0.01 (4.8)	4.71 ± 0.1 (2.1)	4.80 ± 0.1
	Root	3.81 ± 0.2 (17.2)	3.64 ± 0.2 (12.0)	2.77 ± 0.1 (14.8)	3.86 ± 0.1 (18.8)	3.68 ± 0.01 (13.2)	2.80 ± 0.2 (13.8)	3.25 ± 0.1
Leucine	Leaf	7.71 ± 0.2 (9.5)	7.52 ± 0.01 (6.8)	7.11 ± 0.02 (1.0)	7.83 ± 0.1 (11.2)	7.61± 0.02 (8.1)	7.23 ± 0.1 (2.7)	7.04 ± 0.1
	Stem	8.80 ± 0.1 (5.6)	8.63 ± 0.1 (3.6)	8.19 ± 0.02 (1.7)	8.87 ± 0.1 (6.5)	8.69 ± 0.1 (4.3)	8.24 ± 0.2 (1.1)	8.33 ± 0.1
	Root	4.76 ± 0.1 (13.1)	4.54 ± 0.01 (7.8)	4.02 ± 0.02 (4.5)	4.85 ± 0.2 (15.2)	4.60 ± 0.2 (9.3)	4.00 ± 0.1 (5.0)	4.21 ± 0.2
Tyrosine	Leaf	3.87 ± 0.02 (12.2)	3.70 ± 0.2 (7.2)	3.55 ±0.03 (2.9)	3.76 ± 0.01 (9.0)	3.60 ± 0.01 (4.3)	3.49 ± 0.3 (1.2)	3.45 ± 0.13
	Stem	3.92 ± 0.01 (8.9)	3.75 ± 0.03 (4.2)	3.40 ± 0.02 (5.6)	3.85 ± 0.2 (7.0)	3.70 ± 0.1 (2.8)	3.37 ± 0.2 (6.4)	3.60 ± 0.02
	Root	2.95 ± 0.01 (18.0)	2.70 ± 0.01 (8.0)	2.36 ± 0.01 (5.6)	2.80 ± 0.02 (12.0)	2.67 ± 0.01 (6.8)	2.33 ± 0.01 (6.8)	2.50 ± 0.02
Phenylalanine	Leaf	4.56 ± 0.2 (16.6)	4.27± 0.2 (9.2)	3.97 ± 0.2 (1.5)	4.42 ± 0.02 (13.0)	4.18 ± 0.01 (6.9)	3.96 ±0.2 (1.3)	3.91 ± 0.3
	Stem	4.20 ± 0.2 (9.7)	4.01±0.1 (4.7)	3.69 ± 0.1 (3.6)	4.20 ± 0.02 (9.7)	3.98 ± 0.2 (3.9)	3.61 ± 0.01 (5.7)	3.83 ± 0.02
	Root	4.50 ± 0.1 (18.4)	4.11 ± 0.2 (8.1)	3.55 ± 0.2 (6.6)	4.19 ± 0.01 (10.3)	4.07 ± 0.2 (7.1)	3.55 ± 0.01 (6.6)	3.80 ± 0.1

- Results are mean ± SE, n = 3 determinations, P=0.05, numbers in parenthesis are percentage difference
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and simulated acid rain treated sample as a percentage of the control.

b) Stress induced by simulated nitric and sulphuric acid rain on amino acids content of Cucurbita moschata

Simulated nitric and sulphuric acid rain stress on amino acids depicted a trend of significant (P=0.05) increase in leaf samples at all pH concentrations, an increase in stem and root amino acids content at pH 2.0 and 3.0 with a decrease in content at pH 4.0 similar to protein. The quantities of amino acids in leaf, stem and root varied in control as well as in simulated acid rain treated parts. Increase in amino acids of *C. moschata* varied according to plant parts and level of acidity. Highest percentage increase in amino acids occurred at pH 2.0, followed by pH 3.0 and the lowest increase at pH 4.0 when compared to the control pH of 6.0. This trend was observed in all amino acids with the exceptions of aspartic acid, glutamic acids, proline and alanine which showed increased amounts in leaf, stem and root of simulated acid rain treated plants at all levels of acidity compared to the control plants. Percentage increases for leaf histidine (essential amino acid) at pH 2.0, 3.0 and 4.0 were 36.1%, 28.7%, 21.3% for SNAR and 31.7%, 24.3%, 11.8% for SSAR respectively. Percentage increase values for SNAR at pH 2.0, 3.0 were 14.3%, 10.0% for stem, 12.6%, 5.1% for root samples and percentage values decrease at pH 4.0 of 9.4% (stem) and 10.5% (root). Increase in leaf content of another essential amino acid threonine had values of 16.4%, 5.6%, 1.4% and 10.6%, 8.0%, 0.9% at pH 2.0, 3.0 and 4.0 respectively for SNAR and SSAR stress. Increase in threonine content at pH 2.0 and 3.0 in stem and root had percentage increase in values of 19.7%, 18.4% and 49.4%, 21.7% with percentage decrease in threonine content at pH 4.0 of 4.4% and 10.2% for SNAR stress. Associated values for SSAR were 16.3%, 9.4% and 41.3%, 11.9% with percentage decrease at pH 4.0 of 2.5% and 6.4%. SSAR stress led to higher percentage increases in methionine with values of 34.6%, 21.8% compared to values of 24.1%, 13.5%, for SNAR. Stem methionine had higher percentage increase values of 31.0%, 19.8% for SSAR as against values of 19.0%, 16.7% for SNAR. Methionine in root had higher percentage values of 47.0%, 22.9% for SSAR compared to values of 39.8%, 16.7% for SNAR. Non essential amino acids also depicted a similar trend of higher values according to acid levels for leaf in SNAR and SSAR treated, while stem and root had increase in content values at pH 2.0 and 3.0 and decrease in content of amino acids at pH 4.0. Percentage increase in leaf cysteine (non essential amino acid) content values induced by SNAR and SSAR at pH 2.0, 3.0 and 4.0 were 14.3%, 6.0%, 2.4% and13.1%, 3.6%, 2.4% respectively. Cysteine in stem and root had increase in content values of 12.2%, 2.5% and 20.0%, 8.0% at pH 3.0 and pH 4.0 with decrease in content values of 8.8% and 16.0% at pH 4.0 for SNAR stress. While SSAR stress caused percentage increase in content values of 12.2%,

1.3% and 14.0%, 10.0% with a decrease in content values of 12.5% and 12.0% for stem and root at pH 4.0. Corresponding values of 11.2%, 7.9% and 7.5%, 5.1% for stem and root for SSAR treated samples at pH 2.0 and 3.0 and percentage decrease in content values at pH 4.0 of 10.8% and 14.3%. Simulated acid rain stress induced significant (P=0.05) increase in aspartic acid (non essential amino acid) content in leaf, stem and root at all the pH tested. Percentage increase in aspartic acid had values of 42.6%, 20.2%, 2.3% for leaf samples, 46.9%, 30.1%, 19.8% for stem samples and 12.6%, 9.0%, 6.8% for root samples in SNAR at pH 2.0, 3.0 and 4.0. While values of 40.2%, 18.6%, 1.5% obtained for leaf, 46.7%, 27.9%, 18.6% for stem and 11.6%, 8.2%, 4.7% for root at pH 2.0, 3.0 and 4.0 respectively for SSAR stress. Values for other amino acids are shown in Table 2.

Table 3: Stress induced by simulated nitric and sulphuric acid rain on vitamins of *Cucurbita moschata*

Vitamins	Plant part	Nitric acid			Sulphuric acid			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Vitamin A (β-carotene) (μg/dl)	Leaf	198.16±0.01(76.5)	184.56±0.01(64.4)	100.63±0.01(10.4)	191.71±0.01(70.4)	75.29±0.01(32.9)	90.81±0.01(19.1)	112.27±0.01
	Stem	260.22±0.01(33.4)	113.65±0.01(41.7)	146.23±0.01(25.0)	252.30± 0.01(29.4)	97.89 ± 0.01(49.8)	145.26±0.01(25.5)	195.04 ±0.01
	Root	259.73±0.01(40.6)	120.35±0.01(34.8)	151.47±0.01(18.0)	248.51± 0.01(34.6)	100.10±0.01(45.8)	138.49±0.01(25.0)	184.68 ±0.01
Vitamin B_1 (Thiamine) (mg/100 g)	Leaf	0.089 ± 0.01(78.0)	0.026 ± 0.01(48.0)	0.039 ± 0.01(22.0)	0.097 ± 0.01(94.0)	0.032 ± 0.01(36.0)	0.040 ± 0.01(20.0)	0.050 ± 0.01
	Stem	0.091 ± 0.01(33.8)	0.032 ± 0.01(52.9)	0.045 ± 0.01(33.8)	0.097 ± 0.01(42.6)	0.040 ± 0.01(41.2)	0.051± 0.01(25.0)	0.068 ± 0.01
	Root	0.057 ± 0.01(62.9)	0.020± 0.01(42.9)	0.027 ± 0.01(22.9)	0.060 ± 0.01(71.4)	0.023 ± 0.02(34.3)	0.030 ± 0.01(14.3)	0.035 ± 0.01
Vitamin B_2 (Riboflavin) (mg/100 g)	Leaf	0.957±0.01(529.6)	0.125 ± 0.01(17.7)	0.139 ± 0.01(8.6)	0.207 ± 0.01(36.2)	0.124 ± 0.01(18.4)	0.142 ± 0.01(6.6)	0.152 ± 0.01
	Stem	0.236 ± 0.01(38.0)	0.130 ± 0.01(24.0)	0.148 ± 0.01(13.5)	0.255 ± 0.01(49.1)	0.143 ± 0.01(16.4)	0.150 ± 0.01(12.3)	0.171± 0.01
	Root	0.181± 0.01(26.6)	0.123 ± 0.01(14.0)	0.130 ± 0.01(9.1)	0.190 ± 0.01(32.9)	0.131± 0.01(9.1)	0.135 ± 0.01(5.6)	0.143 ± 0.01
Vitamin B_3 (Niacin) (mg/100 g)	Leaf	0.812 ± 0.01(64.7)	0.261± 0.01(47.1)	0.331 ± 0.01(32.9)	0.985 ± 0.01(99.8)	0.299 ± 0.01(39.4)	0.401 ± 0.01(18.7)	0.493 ± 0.01
	Stem	0.992 ± 0.01(58.7)	0.314 ± 0.01(49.8)	0.562 ± 0.01(10.1)	0.999 ± 0.01(59.8)	0.437± 0.01(30.1)	0.570 ± 0.01(8.8)	0.625 ± 0.01
	Root	0.774 ± 0.01(58.9)	0.201± 0.01(58.7)	0.348 ± 0.01(28.5)	0.803 ± 0.01(64.9)	0.236 ± 0.01(51.5)	0.350 ± 0.01(28.1)	0.487 ± 0.01
Vitamin B_5 (Pantothenic acid) (mg/100 g)	Leaf	0.198 ± 0.01(18.6)	0.134 ± 0.01(19.8)	0.147 ± 0.01(12.0)	0.202 ± 0.01(21.0)	0.142 ± 0.01(15.0)	0.155 ± 0.01(7.2)	0.167± 0.01
	Stem	0.196 ± 0.01(17.4)	0.133 ± 0.01(20.4)	0.147 ± 0.01(12.0)	0.201± 0.01(20.4)	0.142 ± 0.01(15.0)	0.156 ± 0.01(6.6)	0.167 ± 0.01
	Root	0.195 ± 0.01(21.9)	0.128 ± 0.01(20.0)	0.131± 0.01(18.1)	0.211 ± 0.01(31.9)	0.141 ± 0.01(11.9)	0.151± 0.01(5.6)	0.160 ± 0.01
Vitamin B_6 (Pyridoxine) (mg/100 g)	Leaf	0.301± 0.01(17.6)	0.220 ± 0.01(14.1)	0.231± 0.01(9.8)	0.321 ± 0.01(25.4)	0.247± 0.01(3.5)	0.250 ± 0.01(2.3)	0.256 ± 0.01
	Stem	0.310 ± 0.01(19.2)	0.224 ± 0.01(13.8)	0.234 ± 0.01(10.0)	0.352 ± 0.01(35.4)	0.249 ± 0.01(4.2)	0.256 ± 0.01(1.5)	0.260 ± 0.01
	Root	0.183 ± 0.01(66.4)	0.081± 0.01(26.4)	0.099 ± 0.01(10.0)	0.198 ± 0.01(80.0)	0.088 ± 0.01(70.0)	0.101± 0.01(8.2)	0.110 ± 0.01
Vitamin B_9 (Folate or Folic acid) (μg/dl)	Leaf	37.06±0.01(12.2)	27.12 ± 0.01(17.9)	30.53 ± 0.01(7.6)	38.09 ± 0.01(15.3)	27.21±0.01(17.6)	31.31± 0.01(5.3)	33.04 ± 0.01
	Stem	37.05±0.01(12.2)	27.10 ± 0.01(17.9)	30.53 ± 0.01(7.5)	37.41± 0.01(13.3)	27.40±0.1(17.06.4)	30.89 ±0.01(6.4)	33.01± 0.01
	Root	11.52 ±0.01(40.7)	6.75 ± 0.01(17.6)	7.82 ± 0.01(4.5)	11.84 ± 0.01(44.6)	6.92 ± 0.01(15.5)	8.00 ± 0.01(2.3)	8.19 ± 0.01
Vitamin K (μg/dl)	Leaf	6.23 ± 0.01(35.7)	5.11 ± 0.01(11.3)	4.63 ± 0.01(0.9)	6.79 ± 0.01(47.9)	5.43 ± 0.01(18.3)	4.73 ± 0.01(3.1)	4.59 ± 0.01
	Stem	6.51± 0.01(44.7)	5.40 ± 0.01(20.0)	4.65 ± 0.01(15.0)	6.66 ± 0.01(48.0)	5.42 ± 0.01(20.4)	4.70 ± 0.01(4.4)	4.50 ± 0.01
	Root	3.90 ± 0.01(63.2)	3.40 ± 0.01(42.3)	2.48 ± 0.01(3.8)	3.98 ± 0.01(66.5)	3.42 ± 0.01(30.1)	2.61 ± 0.01(9.2)	2.39 ± 0.01
Vitamin C (Ascorbic acid) mg/100 g	Leaf	16.03 ± 0.01(18.7)	16.62 ± 0.01(15.7)	17.32 ± 0.01(12.2)	15.77 ± 0.01(20.0)	16.46 ± 0.01(16.5)	17.02 ± 0.01(13.7)	19.72 ± 0.01
	Stem	15.00 ± 0.01(15.0)	16.11 ± 0.01(8.8)	17.20 ± 0.01(2.5)	15.00 ± 0.01(15.0)	16.01± 0.01(9.3)	17.05 ± 0.01(3.3)	17.64 ± 0.01
	Root	9.84 ± 0.01(33.8)	11.56 ± 0.01(22.3)	13.95 ± 0.01(6.2)	9.51 ± 0.01(36.0)	11.21± 0.01(24.6)	13.38 ± 0.01(10.0)	14.87 ± 0.01

- Results are mean ± SE, n = 3 determinations, P=0.05
- Nitric acid = HNO_3, Sulphuric acid = H_2SO_4, Concs. = Concentrations
- Percentage difference values were obtained by expressing the difference between the value for the control and Simulated acid rain treated sample as a percentage of the control.

c) *Stress induced by simulated nitric and sulphuric acid rain on vitamins of Cucurbita moschata*

Results of increase and decrease in vitamins caused by simulated nitric and sulphuric acid rain are presented in Table 3. Screening of leaf, stem and root samples of *C. moschata* revealed the presence of vitamin A, C, K and the B-complex vitamins. The concentration of vitamins in plant parts varied in control as well as in simulated acid rain plant parts. Vitamins A, B_1, B_2 and B_3 in the control were higher in stem than in

leaf and stem, vitamin K and C were more in leaf than in stem and root. Vitamin B_9 did not differ significantly in leaf and stem while vitamin B_5 did not differ statistically in all plant parts. A general trend of highest increase in vitamins at pH 2.0, highest reduction at pH 3.0 and lowest reduction at pH 4.0 in vitamin content of leaf, stem and root of *C. moschata* was observed. Results revealed an increase in vitamin A and the B-complex vitamins at pH concentration 2.0 with a decrease in content at concentration 3.0 and 4.0. However, vitamin C was significantly (P=0.05) reduced by simulated acids rain stress while vitamin K showed increase in content at all levels of acidity. Highest reductions and increases in vitamins occurred at pH 2.0, followed by pH 3.0 and lowest at pH 4.0. Results revealed that simulated nitric acid rain caused more increases and decreases in all the B-complex vitamins and vitamin C while simulated sulphuric acid rain caused more increases and decreases in vitamins A and K. Results of an initial increase in vitamin A content at pH 2.0 had values of 198.16 ± 0.01, 260.22 ± 0.01 and 259.73 ± 0.01 for leaf, stem and root in simulated nitric acid rain, with decrease in content at pH 3.0 and 4.0 with values of 84.56 ± 0.01, 113.65 ±0.01, 120.35 ± 0.01 and 100.63 ± 0.01,146.23 ± 0.01, 151.47 ± 0.01 μg/dl respectively. Corresponding increase in values for simulated sulphuric acid rain of 191.71± 0.01, 252.30 ± 0.01, 248.51± 0.01 μg/dl and decrease in values of 75.29 ± 0.01, 97.89 ± 0.01, 100.10 ± 0.01 (pH 3.0) and 90.81 ± 0.01, 145.26 ±0.01, 138.49 ± 0.01 μg/dl (pH 4.0) for leaf, stem and root compared to control values of 112.27 ± 0.01, 195.04 ± 0.01, 184.68 ± 0.01 μg/dl respectively. Increase in vitamin B_1 content in leaf at pH 2.0 had value of 0.089 ± 0.01 with decrease of 0.026 ± 0.01and 0.039 ± 0.01 for pH 3.0 and 4.0 in simulated nitric acid rain stress. Simulated sulphuric acid rain had increase in value of 0.097 ± 0.01 (pH 2.0) with decrease in content of 0.032 ± 0.01 and 0.040 ± 0.01 mg/100 g. Increase and decrease in vitamin B_1 in stem for SNAR and SSAR had values of 0.091 ± 0.01, 0.032 ± 0.01, 0.045 ± 0.01 and 0.097 ± 0.01, 0.040 ± 0.01, 0.051± 0.01 mg/100 g compared to pH 6.0 value of 0.068 ± 0.01 mg/100 g. Vitamin B_1 in root had values of 0.057 ± 0.01, 0.020 ± 0.01, 0.027 ± 0.01 (SNAR) and 0.060 ± 0.01, 0.023 ± 0.02, 0.030 ± 0.01 mg/100 g (SSAR) respectively in comparison to 0.035 ± 0.01 mg/100 g for the control. Vitamin K content of leaf, stem and root of *C. moschata* was significantly (P=0.05) increased by SNAR and SSAR stress when compared to the control. Increase in mean values for vitamin K of leaf at pH concentrations 2.0, 3.0 and 4.0 were 6.79 ± 0.01, 5.43 ± 0.01, 4.73 ± 0.01 and 6.23 ± 0.01, 5.11 ± 0.01, 4.60 ± 0.01 respectively for SNAR and SSAR in comparison to control value of 4.57 ± 0.01 μg/dl. Increase in stem and root K and vitamins B_2, B_3, B_5, B_6, B_9 and C due to simulated nitric and sulphuric acid rain stress are as shown in Table 3.

VIII. Discussion

All forms of disturbances that causes alteration in the morphology, physiology and biochemistry of the plant is stress. The nutrients quality of *C. moschata* with great medicinal potentials were seriously affected by simulated nitric and sulphuric acid rain stress as revealed in this study. Results suggests that the leaf, stem and root of this *C. moschata* is a rich source of ash, protein, fat, fibre and carbohydrate (proximate nutrients), essential amino acids; histidine, lysine, methionine, leucine, isoleucine, threonine, phenylalanine, valine, tryptophane and non essential amino acids; arginine, aspartic acid, serine, glutamic acid, proline, glycine, cysteine, tyrosine, alanine and vitamins (Vit. A, B_1, B_2, B_3, B_5, B_6, B_9, C and K). From the nutritional point of view high consumption of *C. moschata* has various health benefits. The tea obtain from the leaves are used against stomach inflammation and jaundice. Presently, *C. moschata* is consumed as a vegetable and medicine in many countries like Yugoslavia, Argentina, India, Mexico, the United States, Brazil and Korea and Nigeria. In Korea, the plant is used traditionally to relieve edema during pregnancy and after delivery.

Acid rain has been reported to induce changes in the cellular biochemistry and physiology of the whole plant (Velikova *et al*., 2000). Increase and decrease in contents of phytonutrients occurred at different pH levels and varied according to acidity levels of simulated acid rain. SNAR and SSAR stress caused increase in leaf protein and amino acids content at all levels of acidity with the exceptions of glutamic acid, aspartic acid and proline which significantly increased in all plant parts. Stress is associated with increase or accumulation of compounds in plants. Increase in proline orchestrated by SNAR and SSAR in this study is in line with previous reports (Hare and Cress, 1997; Mofunanya et al. 2009) of proline accumulation in response to a wide range of biotic and abiotic stresses. This accumulation is commonly being encountered in natural environments. Exposure of tomato to simulated acid rain engendered accumulation of soluble phenols as an induced mechanism against SAR stress. These plant nutrients are important; Arginine, carnitine and cysteine significantly improve sperm quality and thus, male fertility. Amino acid cysteine, glutathione and carnitine are powerful antioxidants which protects cells from oxidative stress caused by free radicals therefore, anti-aging effects. Leucine, isoleucine and valine are anticancer agents. Arginine is used as a natural cure for impotence as they fight erectile dysfunction/impotence and improve sexual stamina. Arginine is also used in the management of menopause by reducing hot flushes.

Kumaravelu and Ramanujam (2004) studied the effect of exposure of green gram (*Vigna radiate* L. Wilczek cv. ADT-1 and Vamban to simulated sulfuric

acid rain (SAR) of pH 5.5, 4.0, 2.5 and 7.0 (control). They reported that acid showers at pH 5.5; soluble protein, reducing and total sugars and starch in both cultivars were higher and slowly decreased with increasing levels of acidity. A fluctuation in contents of proximate nutrients imposed by simulated acid rain stress was observed. Kausar and Khan (2009) documented that at pH 4.0 and 3.0 seed carbohydrate and seed protein of wheat plant was suppressed by simulated acid rain. Increase in protein induced by SNAR and SSAR stress is hazardous. Very high protein in vegetables and other foods are unsafe. High protein in foods promotes the intake of protein between 200 and 400 g per day equating to approximately 5 g/kg per day which is more than five times the Recommended Dietary Intake (RDI) (Australian Nutrient Reference Values NRVs, 2006). The problems of very high protein in vegetables and diets are that; very high protein promotes a very low intake of carbohydrates. If the body does not receive enough dietary carbohydrate, it will break down muscle tissue to make glucose (preferred body fuel) which causes muscle wastage, decrease in metabolism and a build-up of ketones. The heart may not function well if its major source of fuel is ketones. The liver and kidneys are put under strain because they have to detoxify and eliminate unusually high quantities of protein byproducts. In people with diabetes, kidney problems may be exacerbated. There is an increased risk of developing gout and gall bladder colic. High protein also causes greater losses of body calcium which increases the risk of osteoporosis. Increase amount of protein in diets can cause mild dehydration due to increased water loss through urine. Increased risk of dehydration puts the body under pressure (Australian Dietary Guideline, 2013). Decrease in protein induced by simulated acid rain stress caused deficiency symptoms to those who eat *C. moschata*. Sypmtoms such as wasting and shrinkage of muscle tissue, the build-up of fluids, particularly in the feet and ankles (oedema), the inability of the body to deliver sufficient amount of oxygen to the cells, usually caused by dietary deficiencies such as lack of iron (anaemia) and slow or gradual growth especially in children.

SNAR and SSAR stress caused increase and decrease in vitamin A and all the B-complex vitamins. However, vitamin C was significantly ($P=0.05$) reduced by simulated acids rain stress while vitamin K showed increase in content at all levels of acidity. These results partly agrees with previous report by Munzuroglu *et al*. (2005) that vitamin levels of plants sprayed with simulated acid rains decreased in respect of pH and time when compared to the control. Several reports on changes in some vitamin levels occasioned by various levels of stress abound (Havaux and Kloppstech, 2001). Mofunanya and Egah, 2017 reported a reduction in vitamin A, B_1, B_2, B_3, B_6, E and C in *Amaranthus hybridus* due SNAR and SSAR effects. Squash is grown in many parts of the world for use as medicine. Squash seeds have been used in Traditional Chinese Medicine (TCM) since at least 17th century. Apart from China, squash has also been used in the traditional medicine of many countries; Yugoslavia, Argentina, India, Mexico, America, Brazil and Korea. *C. moschata* is used in the prevention or control of diabetes and elimination or treatment of intestinal parasites (Caili *et al*., 2006). *Cucurbita moschata* serves as a staple food and doubles as medicinal component in folk medicine used for measles, insomnia, colic and treatment of amoebasis. The seed is vermifuge; it is eaten fresh or roasted for the relief of abdominal cramps and distension due to intestinal worm's parasite. It can also be used in treatment of bladder disorder, wounds and certain female reproductive complaints (Grosvenor and Smolin, 2002). Squash or pumpkin seeds have been used for the treatment of enlarged prostrate glands and parasites (Brown, 2001; Khare, 2003). Fu *et al*. (2006) documented that *C. moschata* possess antidiabetic, antihypertension, immune modulation, antibacteria, antihypercholestrolemia, antitumor, intestinal anti-parasitia, anti-inflammation and antalgic properties.

It is an excellent source of vitamin A carotenoids such as α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin which contribute to *C. moschata* claim as an anti-cancer agent (Bauman and Edwards, 2015). Vitamin A; α-carotene in *C. moschata* slows the aging process, reduces the risk of cataracts development thus, protecting the eye and prevent the growth of tumor. β-carotene reduces skin radiation damage and acts as an anti-inflammatory agent. Carotenoids in *C. Moschata* plant play a role in cancer prevention. One of its best known functions of vitamin A is its involvement in visual pigment formation in the retina of the eye thus, counteract night blindness and weak eye sight, aid in the prevention of macular degeneration, essential in bones and teeth development, builds healthy skin and mucous linings that act as a protective barrier against bacteria and viruses, promotes normal functioning of the male and female reproductive system. Retinol is necessary for epithelial tissues maintenance that lines both the internal and external surfaces, it is a powerful antioxidant that protects against cancer and cardiovascular disease by neutralizing free radicals in cells, required for the strengthening of the immune system against colds, flu and infection, promotes healthy hair and nails, may prevent skin problem like ache, promotes healthy skin free of wrinkle, and help remove eye age spots. Low levels of vitamin A could lead to night blindness, in cases of prolonged deficiency xerophthelmia (dry eyes) which ultimately lead to blindness. The health benefits of *C. moschata* is linked to its antioxidant activity has been reported and authors recommended the consumption of squash for it good taste, as a way to improve the nutritional deficiency of vitamin A and reduce diseases associated with it

(Gonzalez *et al*., 2001). Antioxidant activity of 41.66% was reported Tamer *et al*. (2010), but the squash cultivar was not specified.

Vitamin C content of leaf, stem and root of *C. moschata* is a strong water-soluble antioxidant that protects cells and tissues against damage resulting from toxic chemicals, pollutants and free radicals responsible for degenerate diseases and aging free radicals damage by donating electrons, and by producing other antioxidant like tocopherol (vitamin E) (Kim *et al*., 2012), helps in cancer prevention by blocking the formation of nitrosamines which are potential cancer-causing agents. Vitamin C is important in the synthesis of collagen in the body, a connective protein which act a ''cellular cement'' holding cells and tissues together. It helps the body immune system fight against infections and diseases. The vitamin helps in the absorption of iron in the body needed for the synthesis of red blood cell, also function in the reduction of bad LDL and in the increase of good HDL cholesterol, thereby helping in the prevention of atherosclerosis and diseases of the heart. It lowers high blood pressure, decreases susceptibility to allergens. Vitamin E is a powerful antioxidant that cancels the free radicals that cause tissue and cell damage. It acts as an antioxidant delaying degenerative diseases, protects skin from sun damage and ultraviolet radiation, delay the risk of skin cancer, may lower or delay the risk of cataracts or AMD (Age-related macular degeneration), help in the prevention of brain decline, neurological diseases like Alzheimer's disease, it lowers blood pressure and the body against heart disease and atherosclerosis. Vitamin E is also important in the formation of red blood cells, fertility and reproduction. Decrease the risk of prostrate and bladder cancer. It is an anti-aging vitamin, helps to boost the immune system functions in the body, vitamin E works in synergy with vitamin A to protect the lungs from pollution.

The flesh and seeds of pumpkin are rich in proteins, vitamins (Scarotenoids and tocopherols) (Stevenson *et al*., 2007) and minerals.

Butternut squash is rich in mineral but low in fat and calories. Though low in fat, does contain some healthy fats like alpha-linoleic acid which is a useful omega-3-fatty acid that the body cannot synthesize naturally used as an anti-inflammatory agent (Mateljan, 2007).The butternut squash is rich in complex carbohydrate and low in saturated fat and sodium, it is a good source of magnesium, manganese and potassium, iron, vitamin C, and riboflavin. These minerals and vitamins have both antioxidant and anti-inflammatory effect, regular intake of pumpkin can help to stabilize blood pressure and boost cardiovascular health, vitamin A helps to breakdown homocysteine which is dangerous by-product of metabolism and in protection of colon cells.

Results also revealed the presence of B-complex vitamins important to man. Vitamin B_1 (Thiamine) supports hydrochloride acid production for the digestive system which is critical for carbohydrate metabolism and energy production. Thiamine optimizes brain function and learning capacity, promotes mental alertness and memory, it fights depression, may slow atherosclerosis progression, needed for the formation of blood cell, important for a healthy nervous system, they coordinate nerves and muscles interaction, needed for normal heart, stomach and intestine muscle tone. It may also be associated with reduced risk of cataracts. Deficiency of thiamine causes beriberi or enlarged heart, loss of appetite, breathing difficulty and congestion in the lung, gastrointestinal disorders, causes stunted growth, Crohn's disease, forgetfulness or mental confusion; severe deficiency can result in brain damage and Wernicke-korsakoff syndrome (a form of dermentia), edema. Vitamin B_2 (Riboflavin) is essential for healthy skin, vision and to alleviate eye fatigue, may help to prevent cataracts, it helps in the conversion of carbohydrate to energy and therefore, play a key role in energy production, it is also essential for growth, and body tissues repair, required for the production of red blood cells and antibodies, required for vitamin B_6 and iron absorption, helps maintain the levels of other B vitamins in the body. Lack of vitamin B_2 causes ariboflavinosis; symptoms include cracks in the corners of the mouth or on lips, sore throat, shiny red-purple, inflamed or swollen tongue, sensitivity to sunlight, loss of taste, appetite, anemia, nerve damage (nervousness, irritability or depression), itching or burning eyes or bloodshot. Vitamin B_3 (Niacin) is an important vitamin for the conversion of food to energy. It is required for DNA production in cells, promotes the nervous system functioning, healthy looking skin, functioning of the digestive system, hydrochloride acid production for the digestive system, needed to slow the development of atherosclerosis and reduce the risk of heart attack. Vitamin B_3 may be useful in the treatment of osteoarthritis, may protect against Alzheimer's disease and mental decline related to age. Niacinamide may help in the treatment of age-related macular degeneration (AMD), may prevent diabetes, may reduce low density lipoproteins (LDL) cholesterol in high doses and triglycerides and increase in high density lipoproteins (HDL). Niacinamide deficiency could cause pellagra, a condition characterized by dementia, dermatitis and diarrhea. Other symptoms are dry, cracked and scaly skin, hostility towards others, lassitude (weakness), loss of appetite, indigestion or gastrointestinal disturbances, insomnia, depression, anxiety or mental confusion. Vitamin B_6 (Pyridoxine) is another B-complex vitamin which helps the immune system in the production of antibodies to fight diseases. It helps the body convert tryptophan to vitamin B_3, reduces the risk of stroke or heart attack by controlling

homocysteine level in the blood, reduces symptoms of premenstrual syndrome (PMS) and may aid in the prevention of oxalate kidney stones in women, and in the treatment of carpal tunnel syndrome. Pyridoxine helps in the degradation of fats and carbohydrates for energy production, helps in the regulation of blood sugar levels, it is essential for protein metabolism. needed for new cell formation and growth, promotes healthy skin and mucous membranes, essential for hemoglobin and red blood cell formation, important for the functioning of normal nerve and brain. It may also help in the treatment of depression. Insufficient amounts of pyridoxine could lead to various health conditions such as depression, abnormalities in mood or irritability, nausea or dizziness, chronic fatigue or muscle weakness, migraine headaches, asthma, increased susceptibility to infection, anemia, hypertension, nerve related problems such as convulsion and seizures, numbness of hands and feet, arm and leg cramps. Deficiency of vitamin B_6 also causes skin disorders such as dermatitis (eczema), sores on lips, mouth, inflammations of mucous membrane of the mouth or tongue.

Simulated nitric and sulphuric acid rain stress caused significant reductions and higher amount of nutrients resulting in fluctuation in quantity and quality in *C. moschata*. The increase in phytonutrients may be attributed to stress induced by SNAR and SSAR. Acid rain as well as other factors in the environment cause stress which leads to increase in free radical formation. Kong *et al*. (2000) reported that acid rain caused an increase in oxygen radicals and decrease in protein in their organ. Vitamins reductions in this study may be due to increase in free radical formation in the plant due to acid rain stress, and plant's use of the vitamins and other phytonutrients in order to resist the stress which results in a loss in nutrients. Reductions may also be due to the fact that in autotrophy crops metabolic ways formed by nutrients synthesis are somewhat inhibited by acidic conditions. It is an established fact that various crops exposed to stress change their metabolism. Decrease could also be due to the sensitivity of these nutrients to stress. The accumulation of these chemical constituents in simulated acid rain treated plant parts suggests that their synthesis is stimulated by stress induced by simulated acid rain as it is with other stress factors in the environment.

Cucurbita moschata should be protected from all forms of acidic rain to stabilize its medicinal quality since the poor populace in Nigeria and other developing countries depends on this vegetable crop plant to meet their health needs. Increase and decrease engendered by SNAR and SSAR stress on *C. moschata* nutrients implicated in medical practice should be given attention as too much or too little of these nutrients have great consequences to the health of the consumers of this vegetable. Simulated nitric and sulphuric acid rain affected the medicinal quality of *C. moschata*.

IX. Conclusion

Findings revealed significant increase and decrease in all phytonutrients in leaf, stem and root of *C. moschata* as a consequence of SNAR and SSAR stress. Individually simulated acid rain caused significant alterations in the medicinal quality of this vegetable.

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