



THE INFLUENCE OF WATER STRESS (DROUGHT) ON THE MINERAL AND VITAMIN POTENTIAL OF THE LEAVES OF *OCIMUM GRATISSIMUM* L.

G.G.E. Osuagwu*, H.O. Edeoga, A.N. Osuagwu

Department of Biological Sciences Michael Okpara University of Agriculture, Umudike Umuahia, Abia State, Nigeria

Abstract

The influence of water stress (drought) on the mineral and vitamin potential of the leaves of *Ocimum gratissimum* was investigated. Cultivated *O. gratissimum* plants grown in plastic planting buckets were subjected to mild water stress by irrigating each planting bucket with 500 ml of water once in a week. On the other hand, in the control treatment, each planting bucket was irrigated with 750 ml of water three times in a week. Treatment commenced two months after seed emergence. The leaves of the plants were harvested one month later for analysis. Results obtained indicated that water stress (drought), significantly ($p < 0.05$) reduced the percentage potassium and calcium content of the leaves of *O. gratissimum*. Conversely, water stress led to significant increase ($p < 0.05$) in the percentage concentration of nitrogen in the plant's leaves. Water stress also was found not to have any significant effect in the sodium, magnesium and phosphorous content of the leaves of the plants. The ascorbic acid content of the leaves of *O. gratissimum* was significantly ($p < 0.05$) decreased by water stress. Water stress had no significant effect on the riboflavin, niacin and thiamine content of the leaves of the plants. The reduction in the potassium and calcium content of the leaves of *O. gratissimum* might be due to the mobilization of these mineral elements from the leaves to the roots of stressed plants to serve as osmo- protectants, helping the plants to with stand drought. The increase in nitrogen content as a result of water stress might be related to the mobilization of nitrogen to the leaves for the synthesis of specialized amino acids and proteins to enable the plants to resist the effect of drought. Water stressed plants are known to contain higher amount of protein when compared with unstressed plants. The reduction in the concentration of ascorbic acid in water stressed plants might be due to its breakdown in response to drought. The results obtained were discussed in the light of current literatures.

Key Words: Vitamins, Water Stress, Mineral Elements, *Ocimum gratissimum*.

Introduction

Plants have played important role as source of food and maintenance of good health since ages. The roles of these plants in the maintenance of good health have been documented [14; 15; 31]. WHO [54], had also noted that the majority of the population in the developing countries still rely on herbal medicine to meet their health need. In Nigeria and other African countries, indigenous people traditionally, use a wide range of plants as source of food and medicine. These plants therefore, constitute a reservoir of a wide variety of compounds which show some medicinal and nutritive properties, thus are used as spices, food or medicine [18; 19; 42; 44; 45]. Many of these indigenous plants contain bioactive compounds that exhibit physiological activities against bacteria and other microorganisms and are also used as precursors for the synthesis of useful drugs [37; 51].

The nutritive, vitamin and mineral content of some Nigerian vegetables have been investigated and are

known to be rich sources of carbohydrate, protein, ash, crude fibre, food energy, crude fat, vitamins and minerals [3; 4; 43].

Vitamins are essential in the body as their deficiency adversely affect metabolism of the body [39]. Ascorbic acid is vital in the formation of intercellular substances of the body. Its deficiency leads to weakening of the endothelial wall of the capillaries due to reduction in the amount of intercellular substances [22]. It is also required for the normal healing of wounds and facilitation of the transformation of cholesterol into bile acid in the liver [22; 38]. Riboflavin is necessary for the release of energy from food during respiration and it helps to prevent skin and eye disorder [40; 52]. Niacin is active in preventing the disease pellagra and thiamine inhibits beriberi disease [52].

Minerals are group of essential nutrients which serve a variety of important metabolic functions and are parts of

* Corresponding Author, Email: gbekus2002@yahoo.com

molecules such as haemoglobin, adenosine tri phosphate, and deoxyribonucleic acid [20; 40]. Potassium is necessary for muscular contraction and conduction of impulses; sodium is essential in the conduction of nerve impulses and the maintenance of osmotic balance [40; 55]. Magnesium is essential for the transfer of phosphate group from ATP, proper functioning of some enzymes and necessary for muscular contraction [40; 55]. Phosphorous helps in building of strong teeth and bones, is also a constituent of ATP and nucleic acid and needed in the chemical reaction of respiration [40; 52; 55]. Nitrogen is a key constituent of protein, hence is very essential for growth [52]. Calcium is essential for strong teeth and bones, blood clotting and muscular contraction [40; 52; 55].

The concentration of mineral elements and vitamins, in plants may be influenced by various agronomic and environmental factors such as water stress.

The mineral content of plant parts has been reported to be controlled by water stress. Potassium content is reported to be increased due to water stress [16; 30; 50]. Water stress is also documented to lead to reduction in potassium content of plants [26; 27; 57]. Research reports indicate that water stress results to increased level of sodium in plant parts [30; 47], while Yu *et al.*, [57], observed that water stress caused reduction in the sodium content of the roots of *Robinia pseudoacacia*, which they attributed to decrease in the proportion of inorganic ions in osmotic adjustment and an increase in organic ions. Water stress has been shown to cause significant increase in the calcium content of plants [16; 48]. Conversely, reduction in the concentration of calcium in plant tissues especially the leaves due to water stress is well documented [7; 26; 28; 57]. This apparent reduction in calcium content of the leaves might be related to the reduction in root activity and leaf water potential due to water stress. The concentration of magnesium in plant's parts is reported to be increased by water stress [16; 24]. On the other hand, reduction in magnesium content due to water stress was also reported [7; 57]. Water stress is generally, reported to cause significant reduction in the phosphorous content of plants [7; 27; 48]. However, Inclan *et al.* [24], observed that water stress led to significant increase in the phosphorous content of plants. Research reports have shown that water stress cause increase in nitrogen content of plants [24; 56], while Khalid [27] and Ramoliya *et al.* [48], reported that water stress caused reduction in the nitrogen content of the leaves of plants.

The concentration of vitamins is reported to be affected by water stress. The ascorbic acid content of plants is observed to be reduced by water stress [1; 29;

32], while Rapala- Kozik *et al.*, [49], reported that water stress led to increased thiamine content of plants.

The pharmaceutical values of the leaves of *O. gratissimum* have been widely reported. Their use in the treatment of upper respiratory tract infection, diarrhea, pile, cough, fever pneumonia, surface wound gonorrhea, worm infestation, stomach ache, and others have been documented [2; 14; 21; 25; 33]. The leaves of *O. gratissimum* are also implicated in the blood coagulation and renal function [5; 17]. They are furthermore, used to prepare soup and porridge for women after child birth among the Igbo's of Nigeria [23], and are used as spices for preparation of food [14; 23]. The leaf extracts are used as insect repellent [11; 36].

This research work investigated the influence of water stress on the mineral and vitamin content of *O. gratissimum* and to determine if water stress will cause increase or decrease in the level of these essential chemical substances in view of their importance in the maintenance of good health.

Materials and methods

Plant sample

The seeds of *Ocimum gratissimum* were obtained from a home stead garden in Amaogwu village Bende town, Bende Local Government Area of Abia State, Nigeria. The plant and seeds were identified by the taxonomic unit of the Botany section of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike Umuahia, Abia State. The seeds were raised into seedlings in nursery boxes before they were transplanted into plastic planting buckets. Cultivation of the seedlings was carried out using 20 planting buckets, each filled with 8 kg of sterilized soil. Two treatment level (stressed (non-irrigated)) and unstressed (irrigated)) in ten replicates were used for the study. In the water stress treatment, 500 ml of water was supplied to each planting buckets once in a week to create mild water stress. In the control experiment (unstressed), each planting bucket was supplied with 750 ml of water three times in a week. Treatment started two months after seedling emergence and was carried out for month. Plant leaves were harvested for analysis at the end of the treatment period. The research was carried out in the Green House of the College of Crop and Soil Sciences, Michael Okpara University Agriculture, Umudike Umuahia, Abia State.

Preparation of leaf sample for analysis

The leaf sample was used for analysis due to the fact that the leaves are the part normally used for preparing meals. The harvested leaves were oven dried

using the Selecta model 150- 900 L oven at 65°C. for 24 hours and ground into powder using Thomas Willey milling machine. Powdered samples were stored in sample bottles and kept in a dry place to be used for analysis.

Determination of mineral salt content.

The concentration of the mineral elements (sodium, potassium, calcium, magnesium, phosphorous, and nitrogen) was determined using the wet digestion extraction method described by Udo and Ojuwale, [53], A.O.A.C. [6] and Novozansky et al., [34].

0.2 g of each of the sieved sample was weight in to a 150 ml conical flask; 50 ml of the extraction mixture (sulphuric acid- selenium- salicylic acid) was added to the sample and allowed to stand overnight. The mixture was heated initially at 30°C. for 3 hours and 5 ml of perchloric acid (HClO₄) added. It was then heated vigourously until the digestion is completed. The solution was allowed to cool and filtered using an acid washed filter paper into a 50 ml volumetric and finally made to mark with distilled water.

Determination of the potassium and sodium content.

The potassium and sodium content of the leaves determined using flame photometer.

5 ml of the digested extract was diluted to 50 ml with distilled water and the galvanometer reading taken using flame photometer by selecting the appropriate photocell (K and Na) at each time. Potassium and sodium standards were also prepared and used in the calibration of the equipment and for calculations. The standards were at concentrations of 0ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm.

Determination of the calcium and magnesium content

The calcium and magnesium content of the leaves were determined using the Versenate- Ethyl diaminate tri acetic acid method.

10 ml of the digested extract was pipette into 150 ml conical flask, a pinch of potassium Ferro cyanide, potassium cyanide and hydroxylamine and hydrochloride added. 20 ml of ammonium buffer and a pinch of solochrome black indicator were also added and titrated with 0.02 M EDTA for both calcium and magnesium. The process was repeated, but 10% sodium hydroxide (NaOH) and solochrome dark blue indicator was used for the titration for calcium alone. The data obtained was used for calculations.

Determination of the phosphorous content.

The phosphorous content of the leaves was determined using the yellow calometric method.

5 ml of the extract was pipette into a 50 ml volumetric flask. 10 ml of vamedo – molybdate yellow reagent added and read in the spectrophotometer at 400 nm wave length. Working standards of 0ppm, 2ppm, 4ppm, 6ppm, 8ppm and 10ppm phosphorous standards were prepared and 10 ml of the reagent added and also read in the spectrophotometer measuring the absorbance at 400 nm wave length. The standards were used to plot a curve for the calculations.

Determination of the nitrogen content

The nitrogen content of the leaves was determined using the Kjeldahl method as described by A.O.A.C. [6].

0.2 g of each sample was weighed into the Kjeldhal flask, a tablet of selenium catalyst was added with a little distilled water. 5 ml concentrated sulphuric acid (H₂SO₄) was gradually introduced into the flask and placed on the digester stand to digest. After digestion was completed, it was transferred into a 50 ml volumetric flask with distilled water and made up to mark. 10 ml of the digested extract was pipette into the “Markham” apparatus with 10 ml of 45% sodium hydroxide (NaOH) and allowed to distil into 10 ml 4% hydroboric acid (H₂BO₃).

Determination of vitamin content.

The vitamin content of the leaves of *O. gratissimum* was determined using the methods of Barakat *et al.*, [10] and A.O.A.C. [6].

Determination of the ascorbic acid content.

The ascorbic acid content of the leaves was determined using the method of Barakat *et al.*, [10].

5 g of the sample was weighed into an extraction tube and 100 ml of EDTA/ TCA (2:1) extracting solution were mixed and the mixture shaken for 30 minutes. It was then transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 minutes. It was transferred into 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette into a volumetric flask and 1% indicator was added. It was then titrated with 20% copper sulphate (CuSO₄) solution to get dark end point. The data obtained was used to calculate the ascorbic acid content of the samples.

Determination of the riboflavin content.

The riboflavin content of the test samples was determined using the method of A.O.A.C. [6].

5 g of the test sample was extracted with 100 ml of 50% ethanol solution and shaken for one hour. This was filtered into a 100 ml flask. 10 ml of 5% potassium

permanganate solution and 10 ml of 30% hydrogen peroxide (H_2O_2) solution were added and allowed to stand over hot water bath for about 30 minutes. 2 ml of 4% sodium sulphate solution was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer.

Determination of the thiamine content.

The determination of the thiamine content in the test samples was carried out using the method described by A.O.A.C. [6].

5 g of the test sample was homogenized with 50 ml ethanolic sodium hydroxide solution. It was filtered into a 100 ml flask. 10 ml of the filtrate was pipette into a flask and colour developed by the addition of 10 ml potassium dichromate and read at 360 nm in a spectrophotometer. A blank sample was prepared and the colour also developed and read at the same wave length.

Determination of the niacin content.

The niacin content of the test sample was determined using the A.O.A.C. [6], method.

5 g of the test sample was treated with 50 ml of 1 M sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution were added to the mixture and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml of potassium cyanide solution was added. The mixture was acidified with 5 ml 0.2 N sulphuric acid and the absorbance measured in the spectrophotometer at 470 nm wave length. The data obtained was used to plot the calibration curve.

Statistical analysis

The design for this study was complete randomized design in ten replicates of each treatment. The normal paired t-test at 0.05 probability level was used to analyse the data to determine significant level between treatments.

Results

The results of the influence of water stress on the mineral and vitamin potential of the leaves of *O. gratissimum* are summarized in tables 1-3.

Table 1: The influence of water stress (drought) on the percentage potassium, sodium and calcium content of the leaves of *Ocimum gratissimum*.

Treatment	potassium	sodium	calcium
Irrigated	1.740 ± 0.025	0.460 ± 0.019	3.910 ± 0.035
Non- irrigated	1.500 ± 0.028	0.500 ± 0.023	1.900 ± 0.032
Mean SE	± 0.039	± 0.026	± 0.050

SE = standard error.

There was significant reduction ($p < 0.05$) of the percentage potassium and calcium contents of the leaves of *O. gratissimum* as a result of water stress (table 1). However, water stress significantly ($p < 0.05$) increased the percentage nitrogen content of the leaves of the plants (table 2). Water stress had no significant effect on the percentage sodium, magnesium and phosphorous contents of the leaves of *O. gratissimum* (tables 1 and 2).

Table 2: The influence of water stress on the percentage magnesium, phosphorous and calcium content of the leaves of *O. gratissimum*.

Treatment	magnesium	phosphorous	nitrogen
Irrigated	0.850 ± 0.013	0.280 ± 0.001	3.910 ± 0.035
Non- irrigated	0.820 ± 0.041	0.280 ± 0.015	1.900 ± 0.032
Mean SE	± 0.039	± 0.017	± 0.019

SE = standard error.

The results obtained showed that water stress led to significant reduction ($p < 0.05$) in the ascorbic content of the leaves of *O. gratissimum* and did not have any significant effect on the riboflavin, niacin and thiamine content of the leaves of the plants (table 3).

Table 3: The influence of water stress on the ascorbic acid, riboflavin, niacin and thiamine content (mg/ 100g) of the leaves of *O. gratissimum*.

Treatment	ascorbic acid	riboflavin	niacin	thiamine
Irrigated	191.831 ± 1.810	1.260 ± 0.015	0.180 ± 0.008	0.260 ± 0.011
Non- irrigated	183.940 ± 0.995	1.260 ± 0.012	0.160 ± 0.011	0.280 ± 0.012
Mean SE	± 2.478	± 0.014	± 0.015	± 0.021

SE = standard error.

Discussion

Water stress caused significant reduction in the potassium and calcium but led to significant increase in the nitrogen content of the leaves of *O. gratissimum*. However it did not have any significant effect on the sodium, magnesium and phosphorus contents of the leaves of the plants.

This noticeable reduction in the percentage potassium and calcium content of the leaves was also reported for potassium [12; 26; 27] and for calcium [7; 26; 28; 57]. The reduction of potassium in the leaves of the plants might be due to the mobilization of potassium ion from leaves to the roots in response to water stress to increase the osmotic potential of the sap of the roots to assist the plants to withstand the effect of drought [7; 50; 56]. The reduction in the percentage leaf calcium content of the plants might be related to the reduction of root activity and leaf water potential due to water stress [28].

The nitrogen content of the leaves of stressed plants was 1.66% and that of the unstressed plants was 1.54%. This observed increase in the percentage nitrogen content of the leaves of the plants as a result of water stress may be due to the mobilization of nitrogen to the leaves for the synthesis of special protein in plants as a mechanism to withstand the effect of water stress. Synthesis of special proteins by plants in response to water stress has been documented [13; 46]. Increased nitrogen content in plants in response to water stress has been reported [24; 56]. The above observation tends to agree with those of Ashraf *et al.* [7], who reported that water stress had no significant effect on the sodium content of wheat plants. However, it tends to be in disagreement with the reports of significant reduction in sodium content of plants due to the effect of water stress [7], and those of Martinez *et al.* [30] and Paranchychianakis and Angelakis [47], who observed that water stress increased the concentration of sodium in plant's tissues. The above observations can be explained by the fact that different plant species react differently to the same environmental stress. Research reports also indicate that magnesium and phosphorous contents of plants may be increased or decreased as a result of water stress contrary to the results obtained from the study [8; 16; 24; 27; 57].

Water stress significantly reduced the ascorbic acid content of the leaves of *O. gratissimum*, but did not have any significant effect on the riboflavin, niacin and thiamine content of the plant's leaves.

Reduction in the concentration of ascorbic acid in plant's organs in response to water stress had earlier on been reported by workers [1; 10; 29; 32; 41]. This observed reduction of ascorbic acid content during water stress might be due to its direct destruction by oxygen and derived species during water stress [1]. This in turn helps in plant's drought resistant mechanism.

It could be concluded from the findings of this study that water stress led to reduction in the quantity of essential minerals potassium and calcium and ascorbic acid, which are very necessary for healthy growth and development, thus affecting efficacy and the pharmaceutical values of the leaves of the plants. However, water stress led to increased nitrogen content which is very vital for growth.

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