Effect of Nitrogen on the Nutritional Quality and Microbial Load of Roselle (Hibiscus sabdariffa L)

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Abstract—**The effect of nitrogen concentration on the nutritional quality and microbial load of samples of Hibiscus sabdariffa were studied. Samples of calyces harvested from three different nitrogen levels viz; 0 kg N / ha, 30 kg N / ha and 60 kg N / ha were used. Nutritional composition and the microbial counts of the calyces were determined. Highest significantly (P = 0.01) different crude protein and nitrogen value of 12.06% and 1.93 % were obtained from calyces fertilized with 60 kg N / ha. Ascorbic acid content of the calyces increased with increase in nitrogen levels. For the microbial load analysis, there was no significant influence of nitrogen on the microbial load. However, the least bacteria (2.6 x 10^4 cfu / ml) and fungi (5.68 x 10^5 cfu / ml) counts were obtained from calyces fertilized with 30 kg N / ha. Therefore, inorganic nitrogen fertilizer of up to 60 kg N / ha can be applied to Roselle plant in order to increase some of the nutritional constituents but should not exceed 30 kg N / ha for the purpose of reduced microbial load.**

Keywords—Nitrogen, nutritional quality, microbial load, roselle

I. INTRODUCTION

Roselle also known as Jamaican sorrel (Hibiscus sabdariffa) is a unique species cultivated in many tropical regions for its leaves, stem, seeds and especially calyces which are dried and used to prepare tea, jams, syrup, jellies and beverages [9]. The calyx has gained attention as well as acceptance because of its use in the preparation of a sour, with appealing flavour and wine-red coloured drink, popularly known as "Zoborodo" in the northern part of Nigeria. Roselle is important to the food, beverage and pharmaceutical industry because of its commercial potential as a natural food and colouring agent which can replace some synthetic products [9]. Ethnobotanical information of Roselle plant revealed its diaphoretic, diuretic, uricosuric, antifungal, antibacterial, sedative, mild laxative, antihypertensive, antitussive, hypercholesterolemia treatment, gastrointestinal disorder treatment, liver damage treatment and kidney stone treatment abilities and it also serves as an agent for decreasing the viscosity of the blood, and for treating the after-effects of drunkenness [1]. Roselle is consumed as hot and cold drinks to its uses in folk medicine. The drinks are widely used as diuretic, for treating gastrointestinal disorders, liver diseases, fever, hypercholesterolemia and hypertension [16]. The ripe calyces are used for hot and cold beverages and medicinally it is used as antispasmodic, hypotensive and antimicrobial agent and for relaxation of the uterine muscle [12]. Effect of several agronomic factors on the productivity of Roselle has been reported [6, 8]. Also, the influence of nitrogen on the quality of Roselle fibre was reported by Babatunde et al. [7]. Nitrogen (N) is of special importance to plants as it is one of the major elements that are essential for plant growth and development [18]. Roselle reacts favourably to N application by growing more vigorously. When roselle is grown for its calyces only half of the recommended amount of fertilizer for vegetable is applied because excessive nitrogen encourages vegetative growth and reduces fruit production [11]. However, Okosun [12] reported a positive response of calyx yield to nitrogen application.

This study was done considering that not much work has been done on the relationship between pH, titratable acidity, microbial load and nutritional constituents as it relates to nitrogen application. The objective of this study was to evaluate the effect of increasing nitrogen levels as it affects these attributes in roselle calyces.

II. MATERIALS AND METHODS

A. Sample collection, Preparation and Processing

The Roselle plants were grown in 1999 and 2000 at the Abubakar Tafawa Balewa University research farm site. Details of site, soil, climate, experimental design, agronomic practices involved are contained in Babatunde [6]. Pooled samples of calyxes used were obtained from three nitrogen levels: 0 kg N / ha, 30 kg N / ha and 60 kg N / ha. The samples were hand-picked separately to remove sticks and extraneous materials. The cleaned samples were packed in polythene bags and stored at ambient temperature throughout the period of study.

The samples were dried under a shade and separately pulverized. 4g of each sample were weighed into a beaker and 200ml of distilled water was added and allowed to stand for 30 mins and then filtered through a funnel using a filter muslin paper cloth.

B. Nutritional Analysis

The percentage total nitrogen and crude protein contents were obtained based on principles and procedure described by AOAC [3] and [5] respectively. Perchloric Acid Digestion (Wet Oxidation) of plant materials was used in the determination of the concentration of K, Na, Ca and Mg [4]. The ascorbic acid content of Roselle calyx was determined based on principles and procedures outlined by Moor (1957).

C. Microbial Load Analysis

The microbial analysis was carried on the 4th diluents of each sample for bacterial and yeast count and the results obtained expressed as colony forming units (cfu/ml).

D. Statistical Analysis:

Analysis of variance of data obtained was carried out with the aid of MINITAB computer software and the significant means were separated using the Duncan's Multiple Range Test.

Table 1: Some nutritional constituents and elements of Roselle calyx treated with different nitrogen levels.

Nutritional Content	O kg N/ha	30 kg N/ha	(60kg/ha)	S.E
% Dry matter	96.84a	96.43a	96.04a	0.098
% Moisture	3.16a	3.57a	3.96b	0.050
% Ash	7.97a	8.20a	7.94b	0.035
% Nitrogen	0.91c	1.38b	1.93a	0.126
% Crude Protein	5.68c	8.686b	12.06a	0.786
Minerals				
Ca	81.70a	88.90a	79.80a	1.180
K	67.60b	103.70a	65.30c	5.300
Na	1.70a	1.50a	2.00a	0.062
Mg	54.10a	43.10a	45.60a	1.420

Table 2: Total Aerobic bacterial counts and yeast counts of Roselle calyces treated with different nitrogen levels

Microbial load	Nitrogen levels 0kgN1/ha	30kgN2/ha	60kgN3/ha
Total aerobic bacterial Count(cfu/ml)	2.81×10^4a	2.61×10^4a	3.67×10^4a
Yeast counts(cfu/ml)	6.52×10^5a	5.68×10^5a	7.56×10^5a

III. RESULTS AND DISCUSSION

There were no significant differences in % dry matter and % ash content among nitrogen fertilizer levels. However, application of nitrogen fertilizer significantly affected % N, % crude protein, and concentration of Ca, K, Na and Mg (Table 1). Application of nitrogen fertilizer increased the ascorbic acid content in Roselle calyx but the increase was not significant as presented in Figure 1. The increase in ascorbic acid follows a trend from 0 to 60 kg N/ ha accordingly. Also not affected significantly with increasing levels of nitrogen treatment was pH and titratable acidity. The results in Figure 2 show 60 kg N / ha with the highest count for bacterial and yeast count (3.67 x 104 cfu/ml) and (7.56 x 105 cfu/ml) respectively and the least bacterial count in 0 kg N / ha for bacterial (2.81 x 104 cfu/ml) and yeast count in 30 kg N / ha (5.68 x 105 cfu/ml). However, there was no significant difference in microbial load at all the levels of treatment.

Increasing Nitrogen fertilizer application did not influence % dry matter and % ash content. Ca and K content increased with increasing nitrogen fertilizer application up to 30 kg N / ha after which further addition resulted to no significant differences in content of these elements. Results similar to this was obtained by Makus [13] who reported increase in the K, Fe, Mn and Zn content of the vegetable amaranth (Amaranthus tricolor L). However, Nitrogen fertilizer did not have any consistent effect on the concentration of Na and Mg. Nitrogen content of Roselle calyx increased with increasing level of nitrogen fertilizer from 0 to 60 kg N/ ha. This is expected since nitrogen is known to be one of the essential macronutrients from the soil that is required by most plants [18]. This agreed with the findings of Musa and Ogbadoyi [15] who reported that application of Nitrogen fertilizer increases protein content, carotene, total N and nitrate content of leafy vegetables. The results obtained showed that with increasing level of nitrogen treatment, the crude protein and potassium contents significantly increased. This correlates with the findings of Olaniyi and Ojetayo [17] who showed that nitrogen application increased the protein content of leafy vegetables such as Celosia argentea. The microbial analysis showed no significant difference with increasing levels of nitrogen treatment which is relevant, since the problem of proliferation of microorganism, with increasing nitrogen treatment will not be a problem, unless of course by recontamination from harvesting implements, environment or farmer. The % Moisture obtained from this study is relevant since it comes short of the Standards (12%) required for dried vegetables especially that adopted by importers world over for importation of dried calyx. The low moisture obtained could be responsible for the low yeast count observed. Therefore, it meets possible international standards for export. The ascorbic acid content of Roselle calyx increased but not significantly with increased level of nitrogen fertilizer from 0 – 60 kg / ha. This agreed with the findings of Musa and Ogbadoyi [15] who reported that increase in nitrogen level increased β – carotene content of leafy vegetables.

IV. SUMMARY

Increasing nitrogen levels had statistical significance on the crude protein quality of Roselle. However, it had no significant difference on the microbial load. Considering that "Zobo" drink can become an alternative beverage in Nigeria and a possible export food, it is recommended that nitrogen fertilizers should be applied to Roselle plant to increase both plant yield and protein content.

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