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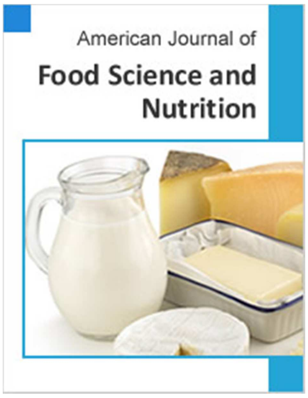

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Proximate Content and Antioxidant Profile of Pumpkin (*Cucurbita pepo* L.) Leafy Vegetable as Influenced by NPK Fertilizer

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Abstract
Pumpkin young shoot, locally called “Gboro” in Nigeria is a palatable and highly nutritious leafy vegetable utilized mostly by the south-western rural dwellers. This study evaluated the influence of NPK fertilizer on the proximate content and antioxidant profile of pumpkin leafy vegetable. The vegetable was raised at the Teaching and Research Farm, Obafemi Awolowo University, Nigeria in 2010 for two seasons and the following NPK fertilizer rates were applied: 0, 50, 100, 150, 200 and 250 kg/ha and replicated six times. Six composite samples from the six replications were analyzed in duplicates for proximate content and antioxidant profile. For the antioxidant assays, crude extract was obtained from cold extraction of each of the samples with 80% methanol and evaporated to dryness. The hydrogen donating or radical scavenging of the extract was determined using the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate). Total phenol content was determined by the method of Singleton and Rossi using the Folin – Ciocalteau reagent in alkaline medium while total flavonoid content was determined using $AlCl_3$ method as described by Lamaison and Carnet. The proanthocyanidin content was determined using a modified method of Porter *et al*, using the AlCl / Butan – 1-0l assay method while the total anthocyanin content of the test samples was determined using the pH differential method of Fuleki and Francis as described by Guisti and Wrolstad. Crude protein, Carbohydrate, Ash, Crude fibre, Ether extract (fat) and Moisture contents were determined using the routine chemical analytical methods of Association of Official Agricultural Chemists (AOAC). The chemical compositions analyzed were reduced significantly when fertilizer rate exceeded 100 kg NPK/ha. The proximate values in pumpkin leaves are 23.8, 0.18, 1.65, and 2.16 g/100g for protein, fat, ash and crude fibre respectively. Fertilizer rates from 150 – 250 kg/ha reduced these values by 10 – 40%. With the increase in fertilizer rates from 100 kg – 250 kg/ha, antioxidant activities was reduced by 19%, phenol by 57% and flavonoid by 65%. For the food value and bioactive compounds of pumpkin to be retained, NPK fertilizer application should not exceed 100 kg/ha if it must be applied.

1. Introduction

Leafy vegetables are the least expensive sources of protective nutrients such as minerals, vitamins, dietary fibre, protein and antioxidants. They could be readily available throughout the year. Consumption of locally available green leafy vegetables is an antidote to overcome nutritional deficiencies [1]. Consumption of vegetables and fruits has been associated with the prevention of chronic diseases such as cancer and cardiovascular diseases [2, 3]. This protective action is attributed to the presence of antioxidants such as ascorbic acid, α-tocopherol, β-carotene and polyphenolic components such as flavonoids, anthochanin and proanthocyanidin in plants [4, 5].

Pumpkin young shoot locally called "Gboro" is a palatable and highly nutritious leafy vegetable utilized in the south-western Nigeria. It contains about 44% protein [6]. The vegetable does not require pre-processing and hot-water blanching unlike many other leafy vegetables consumed in Nigeria.

Traditionally, pumpkins are planted in organic-rich dump sites. However with the change from an agrarian society with a subsistence type of agriculture to a modern mechanized society there is a marked increase in the use of fertilizer to enhance crop productivity [7]. Fertilizers are sources of plant nutrients that are added to soil to supply or enhance the soil fertility. They are intended to supply plant needs directly through modification of such properties as soil pH and structure [8]. There is usually a dramatic improvement in both quantity and quality of plant growth when appropriate fertilizers are added.

Application of fertilizers to soil does not only improve yield but also has significant effect on the quality and bioactive compounds of food crops. Preliminary studies of how NPK influences pumpkin leafy vegetable was carried out in 2007 and 2008 using 0, 90, 180 and 270kg/ha NPK 15:15:15. 180kg/ha was the optimal level observed then both for the yield and the antioxidants concentration [9]. Based on this background, this study was designed to evaluate what effects the fertilizer would have on the vegetable between 90 and 180kg/ha NPK levels. Hence, considering the wide gap between 90 and 180kg/ha NPK levels, six levels: 0, 50, 100 and 150, 200 and 250 kg/ha were introduced to evaluate their effects on the protein, ash, crude fibre, fat, carbohydrate, antioxidant activities and phenolic antioxidant components of pumpkin leafy vegetable.

2. Materials and Methods

2.1. Field Study

Field study was conducted at the Teaching and Research Farm, Obafemi Awolowo Univerity, Ile-Ife, Nigeria for two seasons, (May - August and August – November) in year 2010. Physical and chemical compositions of the soil of the experimental site were determined. The experiment was a randomized complete block design consisted of 6 rates of NPK 15:15:15 fertilizer at 0, 50, 100, 150, 200, 250 kg/ha. The experiment was replicated six times, there were 6 plots per replication and each plot size was 10 m X 12 m and consisted 7 rows. Young shoots of pumpkin biomass was harvested at 6 weeks after planting. The dry matter was obtained by drying the samples to constant weight in the oven at 70°C for 24 hours. The dry matter at 6 weeks was then milled and stored in the refrigerator. Six composite samples from the six replications were analyzed in duplicates for proximate and antioxidant compositions.

2.2. Laboratory Analyses

From the composite samples, 5 g were weighed into different vials and 15 ml each of 80% methanol was added and vortexed. The cold extraction, that is extraction not involving heat, took place for 24 hours. The crude extract was obtained by evaporation of the methanol soluble extract to dryness. The hydrogen donating or radical scavenging of the extract was determined using the stable radical DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) according to the method described by Brand-Williams [10] DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. Total phenol content was determined by the method of Singleton and Rossi [11], using the Folin – Ciocalteau reagent in alkaline medium. Total flavonoid content was determined using $AlCl_3$ method as described by Lamaison and Carnet [12]. The proanthocyanidin content was determined using a modified method of Porter *et al.*, [13], using the AlCl / Butan – 1-01 assay method. The total anthocyanin content of the test samples was determined using the pH differential method of Fuleki and Francis [14], as described by Guisti and Wrolstad [15]. Crude protein, Carbohydrate, Ash, Crude fibre, Ether extract (fat) and Moisture contents were determined using the routine chemical analytical methods of Association of Official Agricultural Chemists (AOAC) [16]. All data were subjected to combined analysis of variance SAS [17]. Means squares, where significantly different, were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

3. Results

Fertilizer influence showed that proximate values of protein, fat, ash and crude fibre in pumpkin young shoot were similar at 0, 50 and 100 kg NPK/ha. The nutrient values reduced significantly when compared to the control from the application of 150 kg/ha of NPK and this continued to the highest fertilizer rate (250 kg NPK/ha). The carbohydrate result was contrary to others. The values increased with increased fertilizer rates. Carbohydrate content in pumpkin young shoot was highest with the application of 200 and 250 kg/ha (Table 1). Seasonal influence showed higher nutrient values during the early season than in the late season except for carbohydrate (Table 2). Variation between the nutrient values range from 3 to 17%. Protein was 8.5% higher in early season while carbohydrate in late season was higher by 3% than in the early season. The influence of season x fertilizer interaction on protein showed that the nutrient concentration was higher across fertilizer rates in the early season (Figures 1). The crude fibre contents of pumpkin young shoot was higher in early season than the late season. The values were significantly lower than in the control when fertilizer application was above 100 kg/ha both in the early and late seasons (Figure 2). The carbohydrate was higher in early season than in the late season. The values were constant with fertilizer rates below 150 kg/ha but this increased sharply at higher rates (Figure 3).

Fertilizer influence showed consistent decreased in values of antioxidant activities and its components as fertilizer rates increased. The control had a significantly higher values than the values from the application of 150 to 250 kg/ha. The addition of 50 and 100 kg gave similar antioxidant values with the zero fertilization at 0.05 probability level (Table 3).

Table 1. *Proximate composition of Pumpkin leafy vegetable as affected by NPK fertilizer levels.*

NPK level (kg ha^{-1})	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Crude fibre (g/100g)	CHO (g/100g)
0	23.8a	0.18a	1.65a	2.16a	72.3c
50	23.6a	0.19a	1.64a	2.16a	72.3c
100	23.7a	0.18a	1.65a	2.15a	72.4c
150	22.4b	0.16b	1.21b	1.15b	75.1b
200	21.3c	0.16b	1.03c	1.12bc	76.5a
250	21.1c	0.15b	0.97c	1.09c	76.6a

Means with the same letter in each column are not significantly different at 5% level of Probability using Duncan's multiple range test.

Table 2. *Proximate composition of Pumpkin leafy vegetable as affected by Season.*

Season	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Crude fibre (g/100g)	CHO (g/100g)
Early season	23.6	0.18	1.49	1.73	73.2
Late season	21.6	0.16	1.23	1.55	75.3
LSD (0.05)	0.45	0.003	0.05	0.02	0.38

NS = not significant at 5% level of probability.
Values are means of duplicate analyses expressed on dry matter basis

Table 3. *Antioxidant activities and its components in Pumpkin leafy vegetable as affected by NPK fertilizer.*

NPK level (kg ha^{-1})	Antioxidant activities (%)	Phenol (mg/100g)	Flavonoid (mg/100g)	Anthocyanin (mg/100g)	Proanthocyanidin (mg/100g)
0	94.5a	32.59a	12.58a	0.138a	0.061a
50	94.5a	32.22a	12.59a	0.135a	0.061a
100	94.9a	32.64a	12.55a	0.136a	0.061a
150	86.3b	21.54b	7.25b	0.094b	0.048b
200	85.7b	18.27bc	5.75c	0.062c	0.039c
250	76.3c	13.96c	4.35d	0.053c	0.039c

Means with the same letter in each column are not significantly different at 5% level of Probability using Duncan's multiple range test.
NOTE: Antioxidant activity and components are presented on this table, so it does not need to be repeated on graphs as requested.

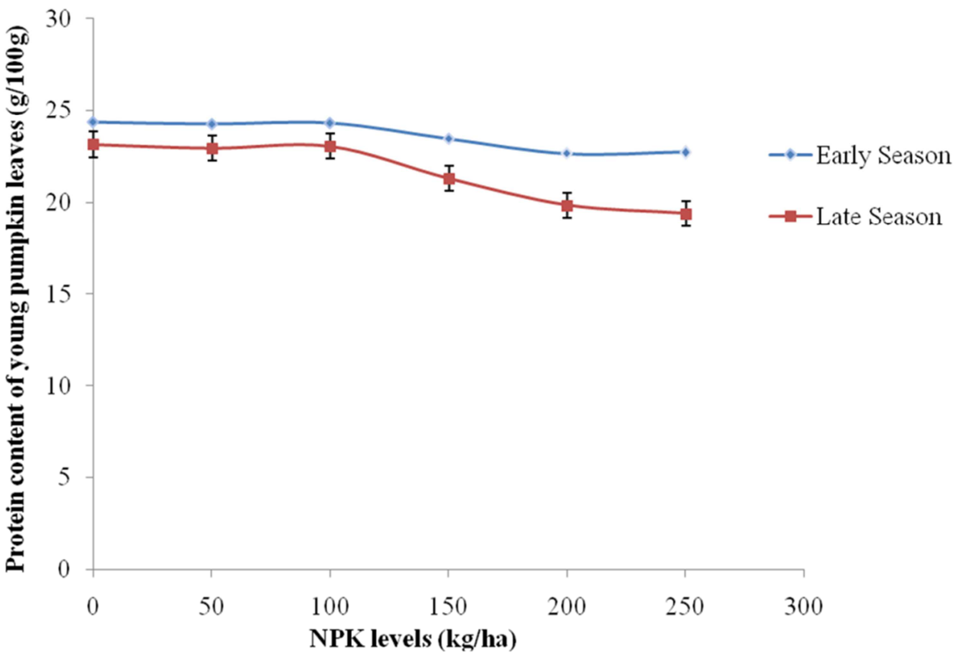

Figure 1. *Protein content of pumpkin leafy vegetable as affected by Season x NPK fertilizer.*

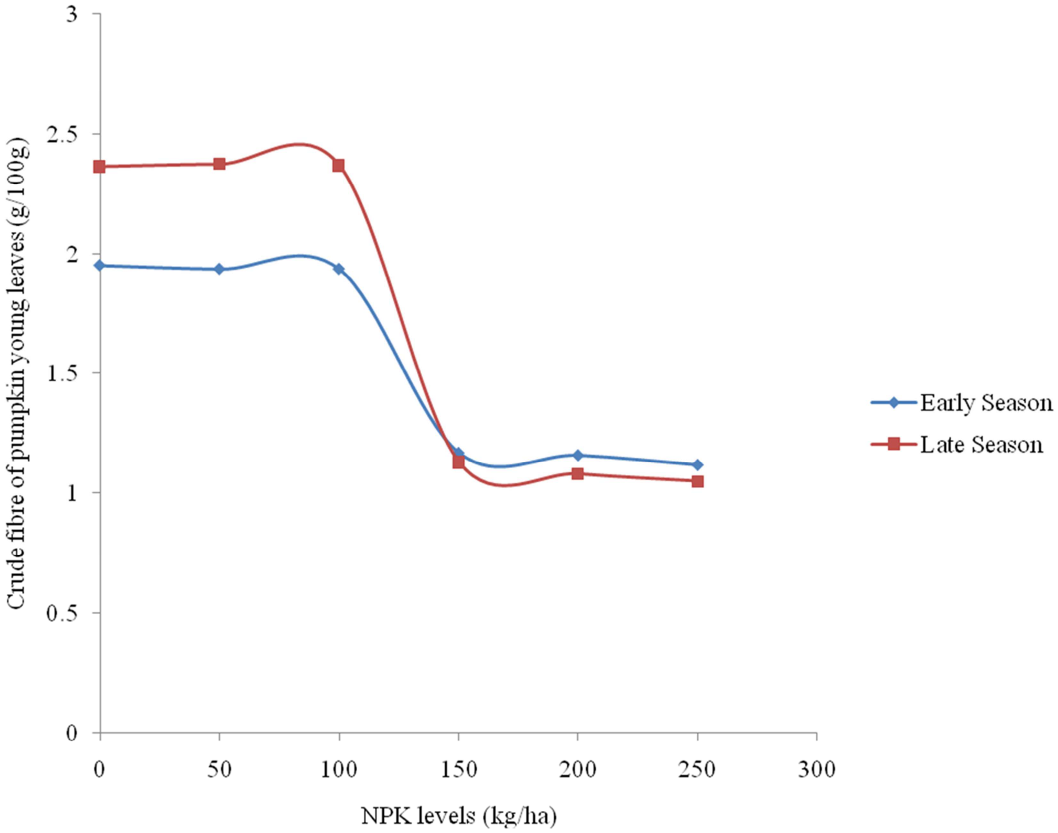

Figure 2. *Crude fibre content of pumpkin leafy vegetable as affected by Season x NPK fertilizer.*

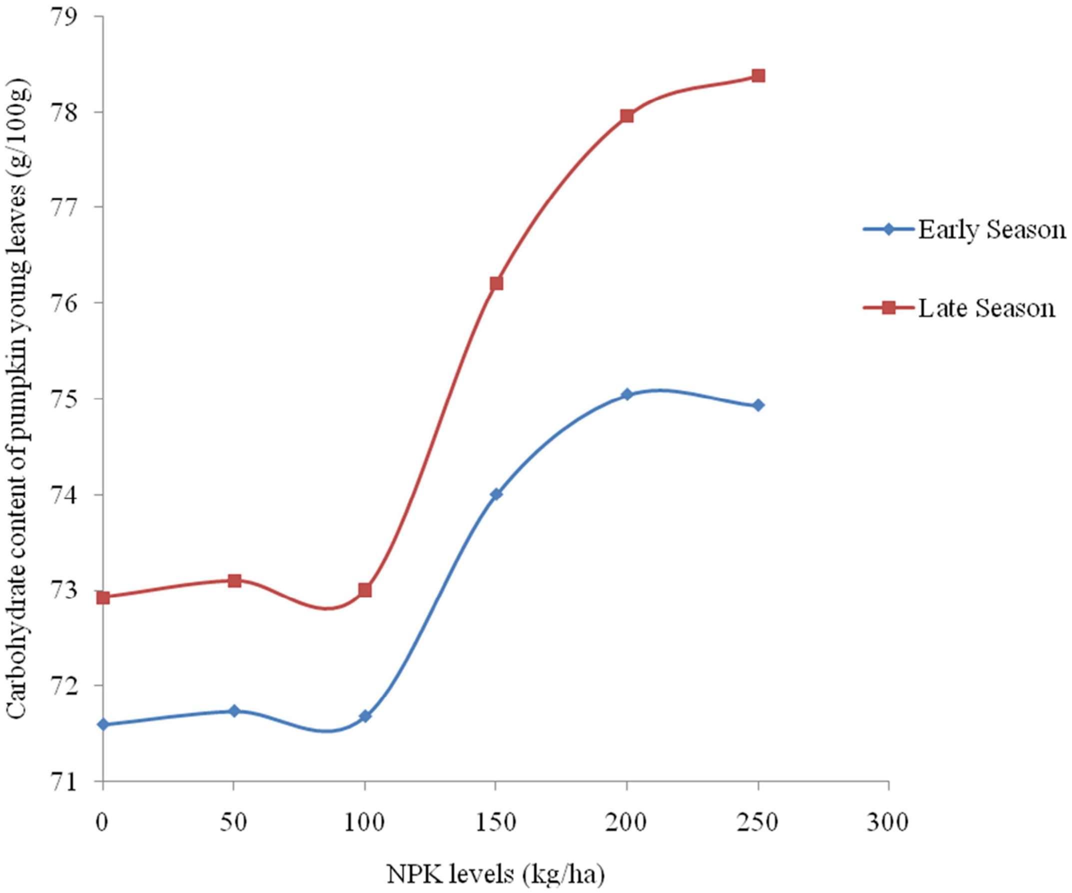

Figure 3. *Carbohydrate content of pumpkin leafy vegetable as affected by Season x NPK fertilizer.*

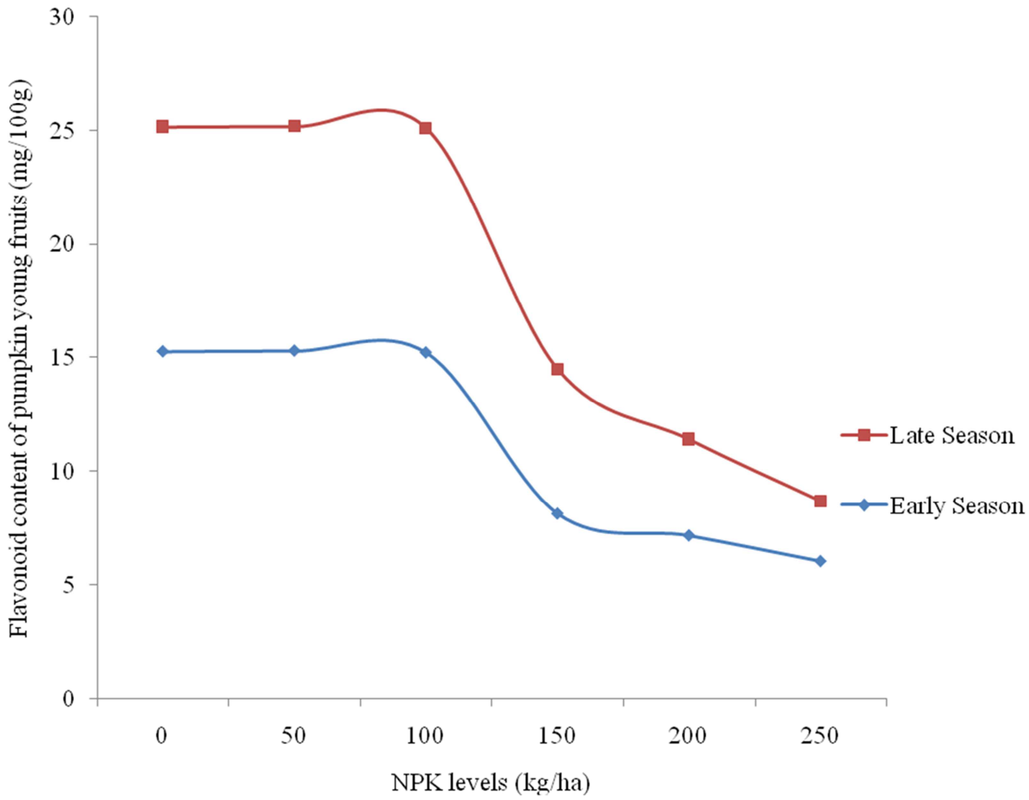

Figure 4. *Flavonoid content of pumpkin leafy vegetable as affected by Season x NPK fertilizer.*

Table 4. *Antioxidant activities and its components in Pumpkin leafy vegetable as affected by Season.*

Season	Antioxidant activities (%)	Phenol (mg/100g)	Flavonoid (mg/100g)	Anthocyanin (mg/100g)	Proanthocyanidin (mg/100g)
Early season	92.2	33.22	11.19	0.11	0.06
Late season	85.2	17.18	7.15	0.09	0.05
LSD (0.05)	1.2	0.89	0.30	ns	0.002

NS = not significant at 5% level of probability.
Values are means of duplicate analyses expressed on dry matter basis

Seasonal variation revealed higher values during the early season in all the determined profiles when compared to the late except for anthocyanin. The values in flavonoid were similar in both seasons (Table 4). The significant (season x fertilizer) interaction on flavonoid concentration showed that early season had higher values than late seasons across fertilizer rates. The concentration at both seasons was similar between the control, 50 kg and 100 kg fertilizer, while significant reduction in values was found at 150 kg/ha fertilizer rate and above (Figure 4).

4. Discussion

A lot of attention has been focused on the use of vegetable and fruits in the supply of protein, vitamins and antioxidants that are of benefit to human health. The protein from pumpkin young shoots range between 21-24g/100g. The protein value at control and 100 kg NPK/ha fertilizer rates (24 g/100g) was higher than at higher fertilizer rates. This value is comparable or higher than some vegetable crops like Amaranth on dry weight basis (5.9g/100g), *Celosia argentea* (5.8g/100g), Cowpea leaves (4.7g/100g), Cabbage (1.6g/100g), Carrot (1.0g/100g), Fluted pumpkin leaves (4.3 g/100g) [18, 19].

The free radical scavenging capacity/antioxidant activities of "Gboro" under control and lower NPK rates was 95%. Low nitrogen has been found to typically results in accumulation of phenolics [20, 21], such as flavonols [22]. This high value is similar to those of *Basella alba* (88.9%), *Crassocephalum crepidioides* (88.9%), *Celosia argentea* (90.2%), *Talinum triangulare* (88.9%), Piper guineese (90.1%) and Amaranthus caudatus (88.9%) [23]. It has been reported that about 29 g of protein is required by adults daily [24], it means that pumpkin leafy vegetable can easily meet the protein required by an adult per day. The plant protein produced by pumpkin leafy vegetable undoubtedly will contribute significantly to the mitigation of protein deficiency in the nutrition of people in developing countries. Though, some essential amino acids are not present in high concentration in plant protein (e.g. Methionine, Lysine, Thiamine), the % crude protein from pumpkin leafy vegetable is still comparable to that of some animal products.

For example, animal protein per 100 g in pork is 14.5 g, Chicken is 20.6 g, Beef is 17.9 g, Egg is 12.1 g while fish (sardine) gives 19.6 g [19].

Early season pumpkin leafy vegetables which received lesser rainfall had higher protein compared to the late season while the antioxidant activities was also higher in the early season. This finding was similar to (*Trichosanthes cucumerina* L.) as observed by Oloyede and Adebooye [25]. Also, Konova and Rainova [26] reported that soybean that received less water had higher crude protein and lower pH since high moisture causes dilution in the cell plasma and this retarded protein forming enzymes and eventually reduce protein synthesis. In addition, Márton [27] reported that excessive rainfall reduced the yield of Rye by 29%.

Beyond 100 kg/ha of NPK fertilizer application, food values and health benefits of pumpkin leafy vegetable reduced. Oloyede and Adebooye [25] found out that there was reduction in fruit proximate composition of snake tomato (*Trichosanthes cucumerina* L.) belonging to the same family of pumpkin- Cucurbitaceae, as the concentration of phosphorous application increased. Increase in biomass due to breeding or improved cultivation methods have decreased nutrients and metabolites in plant. Decreased levels of N have been positively correlated with production of plant defense compounds such as phenolics and flavonoids [28, 29]. The present study confirmed that excessive inorganic fertilizer decreased nutrient composition and antioxidant profile in pumpkin leafy vegetable.

5. Conclusions

The result of this study shows that pumpkin leafy vegetable does not require fertilizer for optimal proximate and antioxidant profiles. However, if fertilizer has to be applied, the application of 100 kg NPK/ha is sufficient for the antioxidant concentration and the proximate content not to be affected negatively. Exploiting this bioactive and protein rich pumpkin at 6 weeks after planting to alleviate national food and nutrition insecurity is paramount.

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