



Nutrient Quality Studies of Fluted Pumpkin (*Telfairia Occidentalis Hook. F*) Leaves as Influence by Fertilizer Microdosing and Micro-Dosing Time

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Authors' contributions

This work was carried out in collaboration between all authors. Author PAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BJA and BAA managed the analysis of the study. Authors OEO, IOA and DJO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The nutrient qualities of vegetables have been noted to be affected by agronomic practices. The study evaluated the effect of fertilizer micro-dosing and time of application on nutrient quality of fluted pumpkin. The field experiment was carried out during 2015/2016 cropping season at the Teaching and Research Farm, Obafemi Awolowo University (O.A.U), Ile-Ife, situated within the forest zone (latitude 07^o28'N and longitude 04^o33'E at 224 m above sea level). Fresh matured

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leaves of fluted pumpkin that have been subjected to four levels of Urea fertilizer which was microdosed on fluted pumpkin were harvested and evaluated for proximate analysis and phyto-chemical screening at the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria. The proximate analysis involved evaluating for percentage carbohydrate, crude protein, ash content, moisture, crude fibre and nitrogen free extract. Also, quantification was made on the same phyto-chemical compound such as anthraquinone, phlobatannins, xanthoproteins, saponins, cardiac glycoside, flavonoids and alkaloid. The experiment was a factorial experiment involving seasons; five levels of Urea fertilizer was micro-dosed at two different times (during planting and two weeks after emergence) and were replicated four times. The experimental design was a 2x4 factorial arranged in a randomized complete block design with four replications. Data was evaluated for proximate and phyto-chemical contents. Data collected were subjected to analysis of variance (ANOVA) and descriptive analysis to determine the effects of fertilizer micro-dosing and time of application on fluted pumpkin leaves and significant means were separated by Duncan Multiple Range Test (DMRT) at (0.05) probability levels. The results showed that, seasons, time of application and the fertilizer micro-dosing significantly affected ($p < 0.05$) the nutrient quality of fluted pumpkin leaf. There were seasonal effects ($p < 0.05$) on fluted pumpkin leaf nutrient quality. For the phyto-chemical analysis, saponins, cardiac glycoside, flavonoids and alkaloid were present for all the treatment applied. Increased fertilizer level decreased the nutrient quality for the proximate analysis and phyto-chemical contents of the plant. Micro-dosing of fertilizer at 40kg N/ha was not significantly different from the effects of 60 and 80kg N/ha on vegetative yield and nutrient component of fluted pumpkin. Hence, 40kg N/ha of micro-dosed Urea fertilizer is adequate for fluted pumpkin leaf nutrient quality.

Keywords: Fertilizer micro-dosing; time of application; fluted pumpkin; nutrient.

1. INTRODUCTION

Generally, leafy vegetables have been found to be of great importance in the supply of necessary nutrients such as vitamins, essential amino acids and minerals in other to aid balanced diet. The production of fluted pumpkin which is one of the major and important leaf and fruit vegetables that can improve the nutritional quality of the increasing population, especially among the urban elite needs to be intensified. Plants are naturally gifted at the synthesis of medicinal compounds, whose characterization has led to discovery of new, cheap drugs with high therapeutic potential [1,2]. Herbal remedies are cheap alternative to conventional medicine. They have contributed significantly to rural livelihood, and apart from traditional healers practicing herbal medicine, many people are involved in collecting and trading in medicinal plants.

Telfairia occidentalis leaf is of high nutritional, medicinal and industrial values which have been found to be rich in protein (29%), fat (18%), minerals and vitamins (20%) [3]. The level of iron is the factor for use of the leaf extraction as blood tonic which can be administered to weak patient [4].

Several agronomic practices such as herbicide, pesticides and fertilizer application have been

found to affect the nutrient qualities of vegetables. Application of nitrogen fertilizer is usually ascribed with the building up of leaf tissues. Plant tissue; usually contain more nitrogen than any other nutrients. Nitrogen application is used to produce rapid vegetative growth of vegetables [5]. It promotes luxuriant growth, increases number of leaves. It is also necessary for reproduction and promotes the uptake of phosphorus and potassium by plants. Since the leaf of *T. occidentalis* are consumed as leafy vegetable therefore there is the need to analyse the leaf of the plant for their nutritional quality in order to ascertain their effect on the nutrient to human health, diet document the optimum rate that will positively increase yield with no detrimental effect on nutritional quality.

Research on the optimum fertilizer Micro-dosing usage for the leave yield is recently being explored. However, information on the effect of fertilizer on nutrient quality of the leaf is limited since little efforts have been made to document the effects of fertilizer on the nutrient component of fluted pumpkin. Soil fertility is a major overriding constraint that affects all aspects of crop production [6]. In the past, inorganic fertilizer was advocated for crop production to ameliorate low inherent fertility in the soils in the tropics. In addition to being expensive and scares, the use of inorganic fertilizer has not

been helpful in intensive agriculture because it is often associated with reduced crop yield, soil acidity and nutrient imbalance [7,8,9,10] reported that soil fertility is one of the main factors affecting the yield of crops. [11] noted that three major nutrient elements known to be deficient in most tropical soils due to intense pressure on land as a result of continuous cropping were N, P, and K. The amount of fertilizer introduced into the soil, no matter the type affects the amount of mineral nutrient available to the plants and the organic carbon content of the soil [12]. Bush fallowing is one of the long aged system used as an efficient, balanced and sustainable agricultural methods for soil remediation, productivity and fertility restoration in the tropics [13], but as a result of rapid increase in the population, the years of fallowing period have been reduced drastically from ten years to three years and this has had an adverse effect on the fertility restoration leading to low and poor crop yield. In developing economies like Nigeria where the population is on the increase, the balanced use of fertilizers becomes imperative to meet the food requirement of the nation [14]. Fertilizer micro-dosing refers to the application of small quantities of fertilizer with the seed at planting time or as top dressing three to four weeks after emergence of seeds [15,16,15] reported that micro-dosing provides sufficient nutrients especially on poor soils or degraded lands in amount that are not too costly and are not too demanding to the environment. Micro-dosing technology was developed and promoted purposively to promote the use of fertilizers in the semi-arid tropics [17,15]. The technology was developed after realising that crop yields in the semi-arid areas of Sub-Sahara Africa have been declining over time due to a decline in soil fertility resulting from mono-cropping, lack of fertilizer, unfavourable climatic conditions and low fertilizer use driven by the belief that inorganic fertilizers burn crops [15]. Micro-dosing fertilizer application increase was found on yield of maize, wheat in East Africa [15] where the rainfall is low compared with Nigeria with heavy precipitation. Micro-dosing technique on production of vegetable crops in Nigeria has not been documented. Fertilizers are important to crops, especially fluted pumpkin for easy nutrient uptake resulting in optimum growth vegetative and seed development.

The recommendation of 160 kgN/ha [18] for the production of fluted pumpkin is observed to be high considering the lost availability and affordability by small scale farmers. Also,

consideration is given to the method of fertilizer application in which majority of the fertilizers are applied as side dressing to the crop thus causing much of the fertilizer to be wasted through run off and evaporation of essential nutrient especially nitrogen.

The need to develop an economically viable technology that will favour sustainable production of *Telfairia occidentalis* among the small holders is the main goal of this work. The effect of fertilizer micro-dosing, level of the fertilizer microdosed and the appropriate time of application as it affect the nutrient composition is being documented by this project.

2. MATERIALS AND METHODS

Fluted pumpkin (*Telfairia occidentalis*) were planted and treated with four levels of Urea fertilizer micro-dosed in late and early seasons of 2015 and 2016 respectively at 0, 20, 40, 60 and 80 kgN/ha. At vegetative growth stage the vegetable stalks were removed and the leaves rinsed with distilled water for phyto-chemical analysis experiment at Department of Biochemistry, Obafemi Awolowo University, Ile-Ife and the fresh leaves were harvested, air dried and grinded into fine powders for proximate analysis at the Institute of Agriculture Research and Training laboratory, Ibadan. The study was conducted at the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife during the late and early cropping seasons of 2015 and 2016 respectively. The experimental design was a 2x4 factorial arranged in a randomized complete block design with four replications.

2.1 Proximate Composition Analysis of Leave Samples

Moisture, crude protein, nitrogen free extract, dry matter, carbohydrate content and ash contents of the leave powder were determined by the standard official methods of analysis of the [19]. Analyses were done in triplicate.

2.2 Phyto-chemical Screening of the Extracts

The components analyzed for were alkaloids, cardiac glycosides, saponins, flavonoids, phlobatanins, xanthoprotein and anthraquinone.

Phyto-chemical screening was carried out on the extract using standard procedures as described

by [20,21,22]. This traced the presence of alkaloids, cardiac glycosides, saponins, flavonoids, phlobatanins, xanthoprotein and anthraquinone in the leave of fluted pumpkin.

3. RESULTS

Effect of treatments on proximate composition of leaf of fluted pumpkin is shown in Table 1. Season had significant effect ($p < 0.05$) on all the parameters measured except percentage nitrogen free extract. The concentration of carbohydrate, moisture and ash were significantly higher in the late planting except dry matter. Time of fertilizer application had no significant effect on the proximate composition of fluted pumpkin.

However, the level of fertilizer applied was observed to significantly affect the composition of the proximate analysis. The proximate

composition increased significantly with increase in fertilizer level from zero to 40 kgN/ha after which they decreased. The peak nutrient composition was obtained at 40 kgN/ha of fertilizer micro-dozing.

3.1 Result of Phytochemical Screening of Leaves Extract of Fluted Pumpkin for the Late and Early Season of 2015 and 2016

The result of qualitative phytochemical screening revealed that distilled water extract of *T. occidentalis* were observed to be present for alkaloid, flavonoid, saponins and cardiac glycoside in all the treatment except treatment 80 kgN/ha that tested negative for saponin. The result obtained indicated that distilled water extract of *T. occidentalis* for all the treatment did not contain anthraquinone, phlobatanins and xanthoproteins. (Table 2 and 3).

Table 1. Mean value showing the combined analysis of variance for the proximate analysis

		CARB%	CP%	MOIST%	NFE%	DM%	ASH%
Toa	Atp	57.45a	15.89a	7.57a	45.76a	91.11a	9.60b
	2 was	60.11a	16.03a	7.83a	46.03a	91.32a	9.96a
	LSD	Ns	ns	ns	ns	Ns	Ns
Season	early	50.50b	15.85a	6.63b	47.22a	91.77a	8.90b
	Late	67.55a	16.07a	8.77a	44.57a	90.67b	10.66a
	LSD	3.34	ns	0.44	ns	0.54	0.34
Fertilizer	0 kgN/ha	58.79a	13.94d	8.11ab	41.15b	88.84d	8.67c
	20 kgN/ha	57.07a	16.23bc	7.41bc	47.52a	90.31c	9.72b
	40 kgN/ha	61.81a	17.36a	8.79a	48.03a	93.27a	11.17a
	60 kgN/ha	58.79a	15.45c	7.26c	46.34a	91.81b	9.54b
	80 kgN/ha	58.69a	16.83ab	6.93c	46.43a	91.85b	9.82b
	LSD	Ns	1.11	0.7	4.62	0.85	0.54

CARB: Carbohydrate(%). CP: Crude protein(%). NFE: Nitrogen free Extract(%). DM: Dry matter(%). ATP: At planting. 2WAS: Two weeks after sowing. TOA: Time of application

Table 2. Result of phytochemical screening of leaves extract of fluted pumpkin for the early season

Phytochemicals	0 kg/ha	20 kg/ha	40 kg/ha	60 kg/ha	80 kg/ha
Anthraquinone	-	-	-	-	-
Phlobatannins	-	-	-	-	-
Xanthoproteins	-	-	-	-	-
Saponins	+	+	+	+	-
Cardiac glycoside	+	+	+	-	+
Flavonoids	+	+	+	+	+
Alkaloid	+	+	+	+	+

+, Present. -, Absent.

The below graph for the effect of fertilizer micro-dosing on the glycosides show early season at planting to be highest at 40 kgN/ha followed by 20 kgN/ha and 60 kgN/ha respectively. Its lowest effect was recorded at 80 kgN/ha and 0 kgN/ha respectively. A significant increase was recorded in the order of 60 kgN/ha, 20 kgN/ha and 40 kgN/ha for early season two weeks after planting while it reduces at 0 kgN/ha and 80 kgN/ha respectively. For late season at planting, the highest increase was recorded at 40 kgN/ha followed by a not-too-wide or slight increase between 80 kgN/ha and 60 kgN/ha with a reduce

recorded at 20 kgN/ha and 0 kgN/ha. At 40 kgN/ha, the highest effect was recorded for late season two weeks after planting with equal increase at 60 kgN/ha and 80 kgN/ha followed by 20 kgN/ha and 0 kgN/ha while 40 kgN/ha shows the most significant increase for all the parameters (Fig. 1).

The highest increase effect of the fertilizer micro-dosing was on the flavonoid of the fluted pumpkin was recorded at 40 kgN/ha for the early season at planting then at 20 kgN/ha and 0 kgN/ha with 60 kgN/ha and 80 kgN/ha having the

Table 3. Result of phytochemical screening of leaves extract of fluted pumpkin for the late season

Phytochemicals	0 kg/ha	20 kg/ha	40 kg/ha	60 kg/ha	80 kg/ha
Antraquinone	-	-	-	-	-
Phlobatannins	-	-	-	-	-
Xanthoproteins	-	-	-	-	-
Saponins	+	+	+	+	-
Cardiac glycoside	+	+	+	-	+
Flavonoids	+	+	+	+	+
Alkaloid	+	+	+	+	+

+, Present. -, Absent.

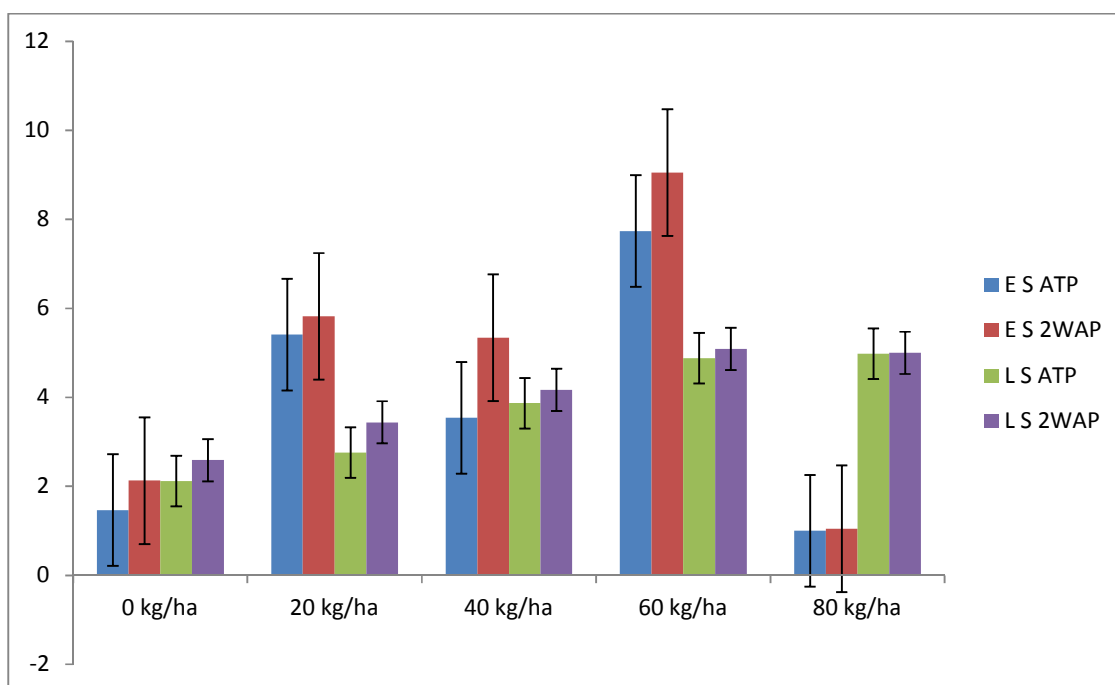


Fig. 1. Effect of fertilizer micro-dosing and time of application on the quantity of glycosides in fluted pumpkin during the late and early cropping season of 2015 and 2016

ES ATP: Early season at planting.
 ES 2WAP: Early season two weeks after planting.
 LS ATP: Late season at planting.
 LS 2WAP: Late season two weeks after planting.

lowest respectively. For early season two weeks after planting has its maximum at 40 kgN/ha followed by 20 kgN/ha and 0 kgN/ha. A reduction in the effect was noticed in the order of 60 kgN/ha and 80 kgN/ha. The increase in the late season at planting were in the order 80 kgN/ha, 0 kgN/ha, 60 kgN/ha, 20 kgN/ha and 40 kgN/ha being the highest. Late season two weeks after planting has its highest at 20 kgN/ha and 40 kgN/ha respectively. 60 kgN/ha has a bit of increase than the 0 kgN/ha with 80 kgN/ha being the lowest. The graph shows the highest and the most significant increase for the four parameters at 40 kgN/ha (Fig. 2).

followed by 20 kgN/ha, 0 kgN/ha with 80 kgN/ha being the lowest. The highest increase was obtained at 40 kgN/ha for the late season at planting. The same level of increase was however observed at 20 kgN/ha and 60 kgN/ha followed by 80 kgN/ha with 0 kgN/ha being the lowest. For the late season two weeks after planting, the highest increase in the effect was recorded at 40 kgN/ha. The increase at 20 kgN/ha not too far from the 60 kgN/h followed by 80 kgN/ha and 0 kgN/ha being the lowest.

The highest level of increase was noticed at 40 kgN/ha throughout the parameters (Fig. 3).

Early season at planting has a sharp progressive increase of alkaloid from 80 kgN/ha to 60 kgN/ha and to 20 kgN/ha with 40 kgN/ha being the highest and 0 kgN/ha, the lowest. A close increase was noticed at 60 kgN/ha and 40 kgN/ha for early season two weeks after planting

Early season at planting shows the lowest increase of saponnin at 0 kgN/ha and 80 kgN/ha at the same level followed by a progressive increase from 20 kgN/ha to 60 kgN/ha and the highest increase at 40 kgN/ha. The highest increase of early season two weeks after planting

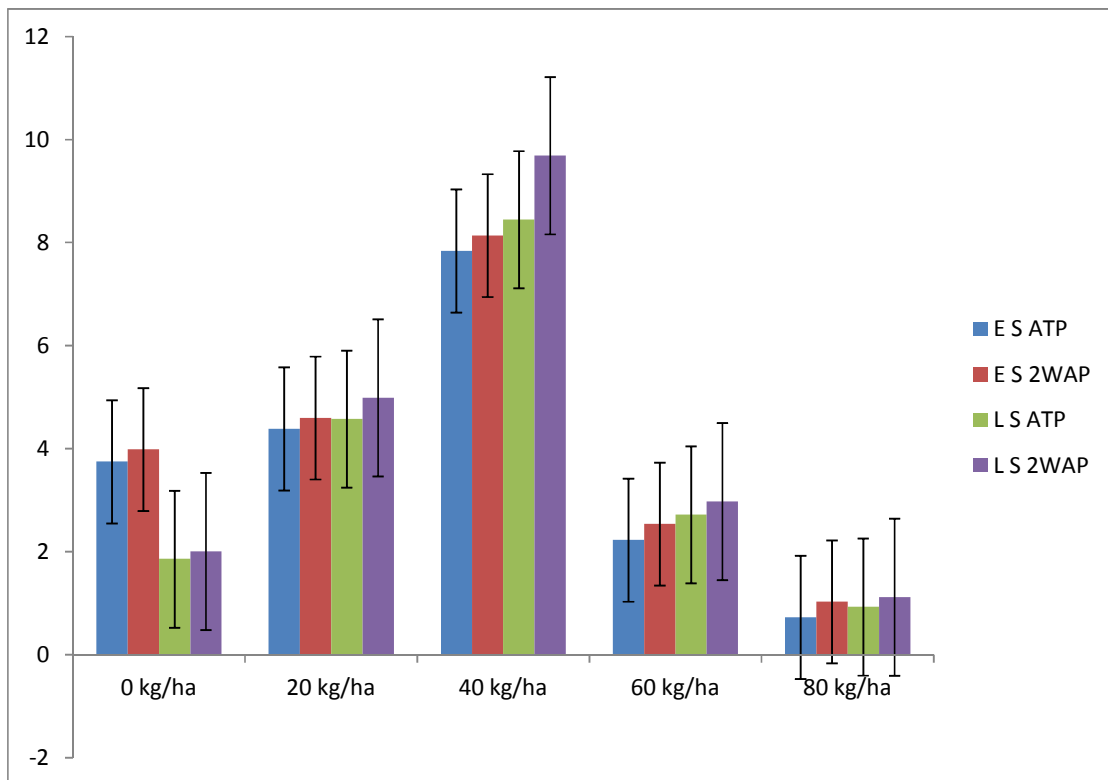


Fig. 2. Effect of fertilizer micro-dosing and time of application on the quantity of flavonoid in fluted pumpkin production during the late and early cropping season of 2015 and 2016

*E S ATP: Early season at planting.
 E S 2WAP: Early season two weeks after planting.
 L S ATP: Late season at planting.
 L S 2WAP: Late season two weeks after planting.*

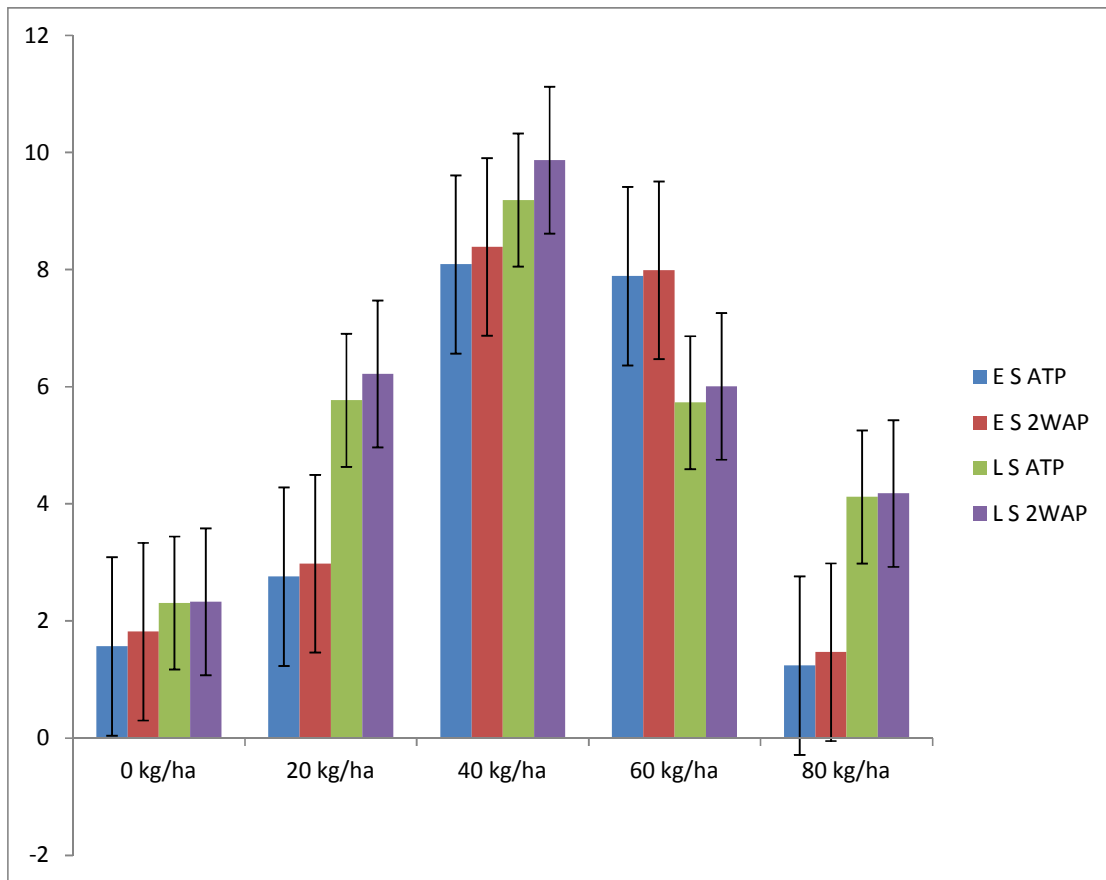


Fig. 3. Effect of fertilizer micro-dosing and time of application on the quantity of alkaloid in fluted pumpkin during the late and early cropping season of 2015 and 2016

E S ATP: Early season at planting.

E S 2WAP: Early season two weeks after planting.

L S ATP: Late season at planting.

L S 2WAP: Late season two weeks after planting.

at 40 kgN/ha with a slight increase between 60 kgN/ha and 20 kgN/ha followed by 80 kgN/ha and 0 kgN/ha being showing the lowest effect. A progressive increase in the order of 0 kgN/ha, 80 kgN/ha, 60 kgN/ha was noticed with 40 kgN/ha having the highest effect for late season at planting. The highest increase for late season two weeks after planting was noticed at 40 kgN/ha then at 20 kgN/a, 60 kgN/ha and 80 kgN/ha respectively. In all, 40 kgN/ha shows the highest increase for all the parameters (Fig. 4).

4. DISCUSSION

The phyto-chemical analysis revealed that aqueous extract of *Telfairia occidentalis* tested positive for the presence of secondary metabolites like alkaloid, saponin, flavonoid, cardiac glycoside, and steroid. A number of

researchers have shown that saponins, alkaloids and a host of other secondary metabolites exhibited anti-inflammatory effects as a result of their membrane stabilizing action on various experimental animal models as reported by [23].

According to [24], saponins can also be used as an inflammatory agent and in treatment for tuberculosis. Saponins was reported to possess the ability to bind cations and other biomolecules in inflammation as such they are able to stabilize the erythrocyte membrane [25].

Alkaloid is a plant-derived compound that is toxic or physiologically active. Some alkaloids such as isopteropodine have anti-microbial activity whereby they act by promoting white blood cells to dispose harmful micro-organisms and cell debris [26]. Alkaloids have been

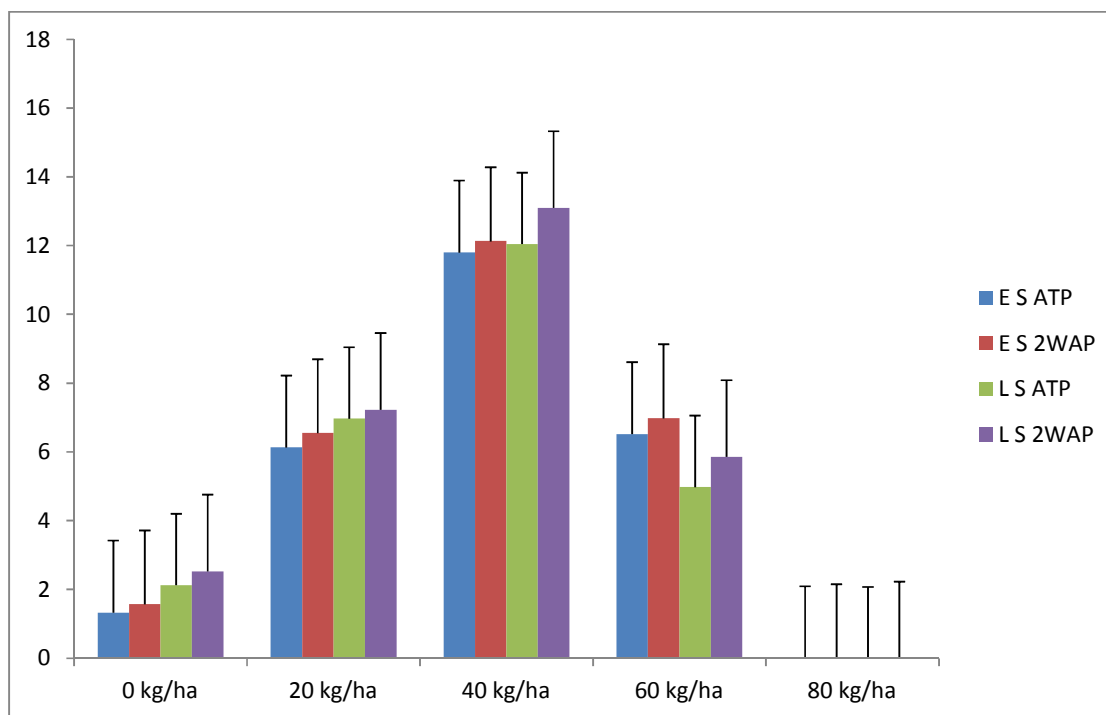


Fig. 4. Effect of fertilizer micro-dosing and time of application on the quantity of saponin in fluted pumpkin during the late and early cropping seasons of 2015 and 2016

E S ATS: Early season at sowing.
E S 2WAS: Early season two weeks after sowing.
L S ATS: Late season at sowing.
L S 2WAS: Late season two weeks after sowing.

reported to be nerve stimulants, convulsants and muscle relaxant, while cardiac glycoside acts as good sedatives and have antispasmodic properties [26]. The presence of cardiac glycosides indicates that they may be potent in curing cardiac insufficiency, coughs and circulatory problems.

Flavonoids constitute one of the most important groups of polyphenols which is responsible for most of the antioxidant activities of plants and plant products and are one of the most diverse and widespread group of natural compounds and it has been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti-inflammatory, and vasodilating actions [27,28]. Thus the distilled extract of the studied vegetable may be a good alternative for the treatment of diseases associated with excessive free radical generation.

Flavonoids can act as an antioxidant because of their ability to scavenge free radicals and to prevent the decomposition of hydroperoxides into

free radicals. The moisture content of the leaves show large variations which could result from environmental factors and management practices.

5. CONCLUSION

It can be concluded that the application of urea fertilizer two weeks after planting performed better compared to urea fertilizer application at planting both for the yield, yield component and nutrient quality for the proximate and phytochemical analysis for the two seasons.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huie CW. Review: Modern sample preparation techniques for the extraction and analysis of medicinal plants. *Journal of Analytical and Bio analytical Chemistry*. 2002;2(3):373,23-30.
2. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Antinociceptive activity of hydromethanolic extract of some medicinal plants in mice. *International Journal of Pharmacy*. 2013;104:120-125.
3. Akanbi WB, Adebooye CO, Togun AO, Ogunrinde JO, Adeyeye SA. Growth, herbage and seed yield and quality of *Telfairia occidentalis* quality as influenced by cassava and mineral fertilizer. *World Journal of Agricultural Sciences*. 2006;3(4): 508-516.
4. Akoroda MO. Ethnobotany of *Telfairia occidentalis* (Cucurbitaceae) among Igbos of Nigeria. *Economic Botany*. 1990;44(1): 29-39.
5. Ojetayo AE, Olaniyi JO, Akanbi WB, Olabiyi TI. Effect of fertilizer types on the nutritional quality of cabbage varieties before and after storage. *Journal of Applied Biosciences*. 2011;48:3322-3330.
6. Mbah CN. Influence of organic wastes on plant *T. occidentalis* growth parameters and nutrient uptake by maize (*Zea mays* L.). *Nigerian Journal of Soil Science*. 2006;16:104-108.
7. Ojeniyi SO. Effects of goat manure on soil nutrients and okra yield in a rainforest area of Nigeria. *Applied Tropical Agriculture*. 2000;5(1):7-12.
8. Ano AO, Agwu JA. Effect of animal manures on selected soil chemical properties. *Nigerian Journal of Soil Analysis*. 2005;15:14-19.
9. Agbede TM. Ojeniyi SO, Adeyemo AJ. Effect of poultry manure on soil physical and chemical properties, Agro I. (Ed.), *Methods of Soil Analysis Part 3 Chemical Methods*. 2nd edition, SSA. 2008;1085-1121.
10. Kolodziej B. Effect of mineral fertilization on ribwort plantain (*Plantago lanceolata* L.) yielding (in Polish). *Acta Agrophysica*. 2006;141:637-647.
11. Aduayi EA, Chude VO, Adebusi BA, Olayiwola SO. Fertilizer use and management practices for crops in Nigeria. Federal fertilizer department, Federal Ministry of Agriculture and Rural Development, Abuja Nigeria. 2002;16.
12. Bijlsma RJ, Lambers H. A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. *Journal of Plant and Soil*. 2000;220:71-87.
13. Ayoola OT, Adeniyi ON. Influence of poultry manure and NPK fertilizer on yield and yield components of crops under different cropping systems in South West Nigeria. *African Journal of Biotechnology*. 2006;5(15):1386-1392.
14. Hera C. Role of inorganic fertilizer and their management practices. *Fertilizer Research*. *International Journal of Soil Science*. 1996;13:63-81.
15. Twomlow S, Rohrbach D, Dimes J, Rusike J, Mupangwa W, Ncube B, Hove L, Moyo M, Mashingaidze N, Mahposa P. Micro-dosing as a pathway to Africa's Green Revolution: Evidence from broad-scale on farm trials. *Nutrient Cycling Agroecosystem*. 2010;88:3-15.
16. Aune JB, Ousman A. Effect of seed priming and micro-dosing of fertilizer on Sorghum and Pearl Millet in Western Sudan. *Experimental Agriculture*. 2011;47: 419-430.
17. Hayashi T, Abdoulaye T, Gerard B, Bationo A. Evaluation of application timing in fertilizer micro-dosing technology on millet production in Niger, West Africa. *Nutrient Cycling Agroecosystems*. 2008;80:257-265.
18. Mangal JL, Batra BR, Singh GP. Studies on nitrogen fertilization under various soil moisture regimes on growth and productivity of melon. *Haryana Journal of Horticultural Science*. 1985;314:232-236.
19. AOAC. Official methods of analysis, Washington DC. Association of official Analytical Chemist. 2000;1058.
20. Sofowora AE. Medicinal plants and traditional medicines in Africa. 2nd edition, Spectrum Books, Ibadan, Nigeria. 1993;289.
21. Harborne JB, Turner BL. *Plant chemosystematics*, Academic Press, London. 2002;123.
22. Tindall HD. *Vegetables in the tropics*. Macmillan Press Ltd., London and Basingstoke. 1968;325-328.
23. David S. Studies force new view on biology of flavonoids. *Journal of Bio Medicine*. 2007;541:737-787.
24. Ong HC. *Tumbuhan liar. Khasiatubatan dan Kegunaanlain*. Kuala Lumpur:

- Utusan Publication and Distributor. 2004;6-9.
25. Oyedapo OO. Studies on the biological activities of the root extract of *Plumbagozey lanica*. International Journal of Pharmacognosis. 2001;34(5):365-369.
26. Ogunwenmo KO, Idowu OA, Innocent C, Esan EB, Oyelana OA. Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. Journal of Biotechnology. 2007;20:2400-2405.
27. Parajuli S, Tilja Pun, Parajuli N, Jamakattel-pandit N. Antioxidant activity, total phenol and flavonoid contents in some selected medicinal plants of Nepal. JHAS. 2012;2(1):27-31.
28. Pereira DM, Valentae P, Pereira JA, Andrade PB. Phenolic: From chemistry to biology. Molecules. 2009;14:2202-2211.

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