

Consequences of X-Ray Irradiation on the Proximate Levels of Fluted PUMPKIN (*Telfairia occidentalis* Hook.F) LEAF.

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Abstract-

A research was conducted at the National Soil Science Laboratory Research Farm of Federal Ministry of Agriculture and Rural Development Umuahia, Abia State, to probe the consequences of x-ray irradiation on the proximate levels of fluted pumpkin (*Telfairia occidentalis* Hook. F) leaf in M_1 and M_2 generations. The fluted pumpkin seed treatments include 0.00 mGy, 6.75 mGy, 10.08 mGy, 14.08 mGy and 18.75mGy. Treated seeds were planted in a randomized complete block design (RCBD) with five replications to raise the M_1 and M_2 generations. Fresh leaves were harvested six (6) weeks after planting and analyzed for proximate levels in both generations. There was a significant ($P<0.05$) decrease in the level of crude protein, moisture content and carbohydrate contents as the treatment concentration increased in both generations. At 10.08mGy treatment dose, a significant ($P<0.05$) increase in fat level was recorded as crude fiber and ash levels also increased at 14.08mGy and 6.75mGy respectively. Though not significant ($P>0.05$). This is a clear cut that increased dose of x-ray irradiation reduces the level of crude protein, moisture and carbohydrate in fluted pumpkin whereas crude fiber, fat and ash levels can be enhanced with 14.08mGy, 10.08mGy and 6.75mGy x-ray treatment respectively.

Key Words: Analysis, concentration, consequences, *Telfairia occidentalis*, x-ray, proximate, mutation.

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I. Introduction:

Induced mutations which are alterations in the genetic makeup of an organism due to interaction with mutagens have been found to successfully create variability and produce new desirable mutants which are multiplied and put to use after vigorous selection and breeding to homozygosity. The frequency of induced mutation is 10-3 gametes against 10-6 gametes in the case of spontaneous mutation and this has made the induced mutation preferable to spontaneous mutation (Muller, 1927).

This research was stemmed by the need to increase variability and availability of fluted pumpkin leaves for human nutritional and medicinal uses as to close the gap created by increase in demand due by increasing population.

Physical mutagens like x-rays and gamma rays have been used to induce plant traits such as nutritional attributes, disease resistance, yields, and height (Iwo *et al.*, 2013). It allows for the induction of the desired characteristics that are either not exhibited naturally or lost during the evolutionary passage. Previous studies (Akpaniwo *et al.*, 2015) revealed that the seed of fluted pumpkin when exposed to high doses of X-rays reduced germination and survival rates of pumpkin. Also, (Al-Enezi *et al.* 2012) revealed significant retardation in seed germination and survival rate in date palm. Hameed *et al.* (2008) and Al-Salhi *et al.* (2004) has found that high exposure to gamma rays disrupt protein synthesis, leaf gas exchange, hormonal balance, and activities of enzymes in seeds.

Fluted pumpkin (*T. occidentalis*) is a perennial plant with coiled tendrils and bifids that enables it in climbing. They belong to the family Cucurbitaceae. The fruits are noted by 10 observable longitudinal ridges and are known to be the largest known (16-50 cm length, 9 cm diameter). The seeds are big, non-endospermic, and normally dark crimson in color, and according to Akoroda, (1990), are lodged within a bright-yellow fibrous endocarp. In different countries and languages, *T. occidentalis* is known as follows: Telfairia nut, fluted gourd, fluted pumpkin, oil nut, and oyster nut, (English); Oroko, pondokoko and Gonugbe (Sierra Leone); Krobonko (Ghana); Costillada (Spanish); Ugwu (Igbo-Nigeria), Ikong (Efik/Ibibio-Nigeria) and Aworoko, Eweroko (Yoruba-Nigeria) (Gbile, 1984).

Fluted pumpkin (*Telfairia occidentalis*) is a vegetable crop consumed for its high nutritional and medicinal value namely: High oil content (30%) (Akoroda, 1990). The Shoots of *T. occidentalis* contain a high amount of iron and potassium, while the seeds are composed of 27% and 53% crude proteins and fats respectively (Aiyelaagbe *et al.*, 2002). High concentration of antioxidants on the leaves (Nwanna *et al.*, 2008). High level of fat and protein in seed. The leaves possess high level of carbohydrates, iron, copper, potassium, and manganese (Idris, 2012). Its medicinal benefits include: anti-microbial functions (Oyewole *et al.*, 2012), anti-diabetic functions (Stanaway *et al.*, 2000), treatment of infertility (Akang *et al.*, 2010), hematological and anti-malaria functions (Okokon *et al.*, 2009).

Following the huge economic, medicinal and nutritional weight of fluted pumpkin, it's of no doubt that its demand has outweighed its variability, availability and supply. To close the demand space, mutation breeding which is a source of increasing variability and improvement without significantly altering acceptable phenotype is needed to supplement conventional breeding (Ojomo *et al.*, 1979).

In this research, five different x-ray doses (0.00 mGy, 6.75 mGy, 10.08 mGy, 14.08 mGy and 18.75mGy) were used to treat five different sets of fluted pumpkin by exposure to x-ray irradiation.

The objectives of this research were to establish the consequences of x-ray irradiation on the proximate level in fluted pumpkin leaves and also to determine the x-ray treatment dose which increases a particular nutrient in fluted pumpkin leaves.

II. Materials And Method

The research was carried out at the National Soil Science Laboratory Research Farm of Federal Ministry of Agriculture and Rural Development Umuahia, Abia State Nigeria. The site is located within longitude 07°33E and latitude 05°29N with altitude of 122m above sea level. Umuahia is a humid tropic with total average rainfall of about 286mm/annum and classified as sandy loam ultisol (Agboola, 1979).

The fluted pumpkin pods were obtained from the National Agricultural Seed Council of National Root Crop Research Institute Umudike. The pods were cut open and the fluted pumpkin seeds extracted.

Treatment of the fluted pumpkin seeds with different doses of X-ray irradiation were carried out at Mecure Diagnostic Center of Abia State Specialist Hospital, Umuahia.

Five samples of twenty seeds each were treated with different doses of X-ray irradiation (0.0 mGy, 6.75 mGy, 10.8 mGy, 14.8 mGy and 18.75 mGy) respectively using a therapeutic medical X-rays device (Clinac 23EX Linear Accelerator, Varian Medical Systems, USA).

The field for planting was properly cleared, ploughed and harrowed to fine tilt, beds were made and planting was done on March 20th 2018 to raise the M₁ generation and March 1st 2019 to raise the M₂ generation.

The experiment was designed in randomized complete block with five replications. The plot size was 2m x 2 m with 0.5 m apart. The planting distance was 120 cm x 60 cm with 2 seeds sown per hole and later thinned to one. All cultural practices such as staking/weeding and fertilizer application were carried out two weeks and four weeks after planting respectively. Six (6) weeks after planting, some portion of the fresh leaves were harvested from all treatment and analyzed for proximate levels while some were left to grow to full maturity and seeds were harvested and used to raise the M₂ generation whose fresh leaves were also harvested after six (6) weeks of planting and analyzed.

Proximate analysis was carried out at O-J Laboratories, Umudike, Abia State.

Data were generated for proximate analysis on the different treatments, based on the following:

• Crude protein percentage

The protein was determined using a micro-kjeldahl method as described by (AOAC, 2005). The protein value was obtained by a conversion factor, and the result expressed as the amount of crude protein.

$$\% \text{ CP} = \% \text{N}_2 \left[\frac{(100 \times \text{N} \times 14 \times \text{V}_f \text{T})}{\text{W} \times 1000 \times \text{V}_a} \right] \times 6.25$$

Where:

W=weight of sample analyzed

N =concentration of H₂SO₄ titrant

V_f=Total volume of digest

V_a=Volume of digest distilled

T=titre value- blank.

CP= Crude protein

• Crude fibre percentage

Fibre content was determined using the method of (AOAC, 2005). The crude fibre content of each sample was calculated gravimetrically as;

$$\% \text{ CF} = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100$$

Where:

W₂= weight of crucible + sample after washing and drying in oven

W₃= weight of crucible + sample ash.

CF=Crude fibre

- **Fat percentage**

Fat content was determined using the method of (AOAC, 2005).

The percentage fat content was calculated as follows:

$$\% \text{ Fat} = \frac{\text{Weight of the extracted}}{\text{Weight of the sample}} \times 100$$

- **Ash content**

Ash content was determined using the method of (AOAC, 2005).

Percentage ash was calculated using the formula: $\frac{W_2 - W_1}{W_1} \times 100$

Where;

W= Dry weight of food sample,

W₁= weight of crucible,

W₂= weight of crucible and ash

- **Moisture content percentage**

Moisture content was determined using the method described by Association of Official Analytical Chemist (AOAC, 2005).

The moisture content was calculated as follows:

$$\% \text{ MC} = \frac{\text{Change in weight}}{\text{Initial weight of sample before drying}} \times 100$$

- **Carbohydrate percentage**

The carbohydrate content was calculated by difference as the nitrogen free extractive (NFE), a method separately described by James (2005).

The nitrogen free extractive will be calculated as

$$\% \text{ NFE} = 100 - \% (a+b+c+d+e)$$

Where; a = protein

b = fat

c = fiber

d = ash

e = moisture.

III. Result

Figure 1 below shows a bar chart representation of the effects of different doses of x-ray treatment on crude protein, crude fiber, fat, ash, moisture content and carbohydrate levels in fluted pumpkin leaves during M₁ and M₂ generation.

Crude Protein:

The bar chart shows a gradual reduction in the level of crude protein as the treatment concentration was increasing. This trend was observed in both M₁ and M₂ generations.

From the result obtained, it was observed that the mutagenic effect on the crude protein was significant (P<0.05) during the M₁ generation. Treatment 0.00mGy recorded the highest percentage of crude protein, followed by 6.75mGy treatment and downwards where treatment 18.75mGy gave the lowest percentage.

Similarly, in M₂ generation, the result of the analysis of variance indicated that the effect of the x-ray doses on the crude protein was highly significant (P<0.05) when compared to the control. From the result, it was also observed that increase in the x-ray doses resulted to a gradual decrease in the protein content of fluted pumpkin. The lowest dose (6.75mGy) recorded the protein content (18.60%) amongst the treatment levels followed by 10.08 mGy (18.12%) and 14.08 mGy (17.95%) whereas 18.75 mGy recorded 17.10% protein content. The controlled plant however gave the highest protein content level (18.85%).

Crude Fiber:

Highest percentage of crude fiber (5.17%) was recorded on 14.08mGy x-ray dose treatment, followed by 10.08mGy dose treatment which gave 5.15%. The untreated (0.00mGy) gave 5.10% which was higher than treatment 18.75mGy dose (5.00%).

However, analysis of variance shows that there was no significant difference ($P>0.05$) based on the different treatment doses.

In M_2 generation, there was a gradual continuous decrease in crude fiber percentage as the treatment concentration increased. Analysis of variance also showed that there was no significant difference ($P>0.05$) on the effects of the treatment.

Fat:

In the M_1 generation, treatment dose of 10.08mGy gave the highest fat percentage of 1.88%. This was followed by 6.75mGy treatment dose which gave 1.82%. Next was the untreated sample which gave 1.77% followed by dose 14.08mGy (1.73%) and 18.75mGy (1.63%).

Analysis of variance shows a significant difference ($P<0.05$) on the effects of the different treatment doses.

In M_2 generation, there was a reduction in the fat percentage as treatment concentration was increasing. The highest among the treated samples was at 10.08mGy (1.82%) which was still lower than the percentage of the control sample (1.89%).

Analysis of variance shows a significant difference ($P<0.05$) on the effects of the mutagen at different doses.

Ash:

Treatment dose 6.75mGy gave the highest percentage of ash (8.22%), this was followed by treatment dose 10.08mGy (8.02%).

However, the control (0.00mGy) gave 7.96% which was higher than doses 14.08mGy (7.45%) and 18.75mGy (7.21%).

Analysis of variance showed that there was no significant difference ($P>0.05$) on the effects of the different treatment doses in M_1 generation.

In M_2 generation, there was a highly significant difference ($P<0.05$) on the effect of the different treatment doses. As the treatment concentration increased, the ash percentage decreased.

Moisture content:

Analysis of variance in both generations showed that there was a highly significant difference ($P<0.05$) on the effects of the different treatment doses.

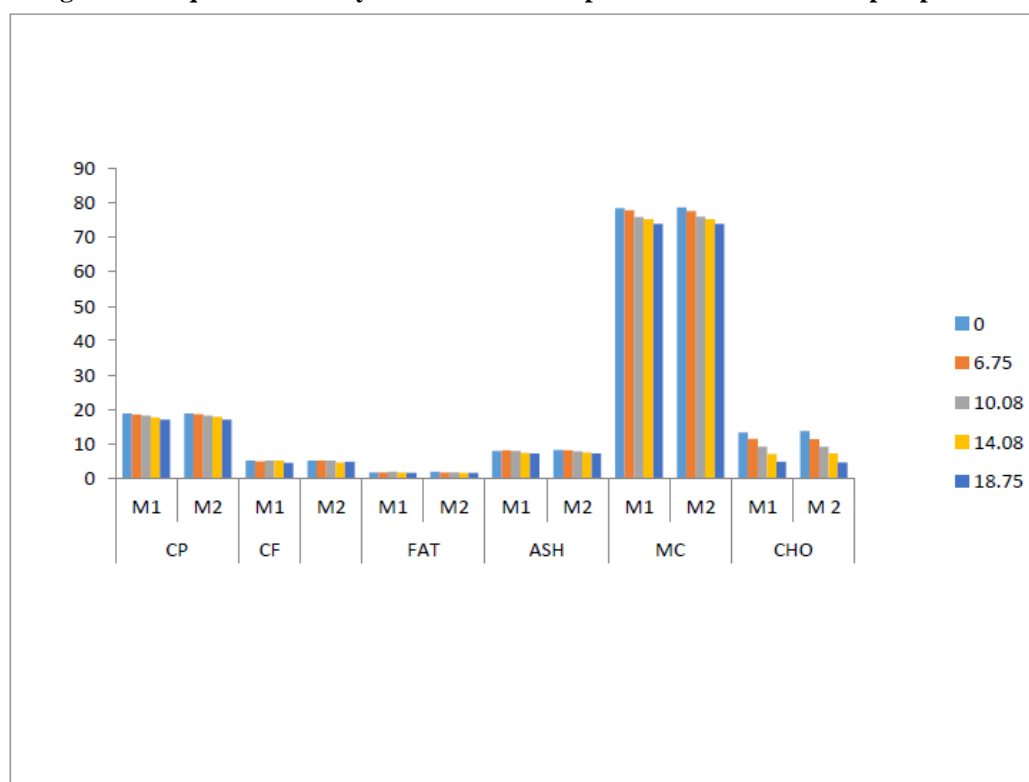
As the treatment concentrations were increasing, the moisture content reduced. This was recorded during M_1 and M_2 generations.

Carbohydrate percentage:

In both generations M_1 and M_2 , there was a trendy decrease of carbohydrate percentage as the treatment doses increased.

This result shows a highly significant difference ($P<0.05$) on the effects of the different treatment doses as revealed by analysis of variance. 13.39% (0.00mGy) was the highest carbohydrate percentage that was recorded while 4.80% (18.75mGy) was the lowest recorded percentage during the M_1 generations. 13.76% (0.00mGy) was the highest carbohydrate percentage that was recorded while 4.66% (18.75mGy) was the lowest recorded percentage during the M_2 generation.

Fig 1. Consequences of x-ray irradiation on the proximate level of fluted pumpkin leaf.



CP= Crude protein, CF= Crude Fiber, MC= Moisture content, CHO= Carbohydrate

IV. Discussion

This research which aimed at investigating the consequences of x-ray radiation on the proximate level in fluted pumpkin leaves has revealed that crude protein, moisture content and carbohydrate level of the fluted pumpkin leaf were reduced by increase in the x-ray doses. The highest percentage of proximate levels were obtained at treatment 0.00mGy. This shows the destructive (alteration) potentials of x-ray irradiation on the nutrient metabolic oxidation pathway and biochemical processes (Younis *et al.* 1962). However, at 10.08mGy treatment dose, a very significant increase ($P < 0.05$) in fat level was observed (1.88). At 14.08mGy and 6.75mGy treatment doses, there were increase in crude fiber and ash levels respectively above other treatments though not significant ($P > 0.05$).

V. Conclusion

The present investigation was conducted to investigate the mutagenic effects of x-ray doses on proximate compositions of fluted pumpkin. Findings from this study showed that increased dose of x-ray irradiation reduces the level of crude protein, moisture and carbohydrate in fluted pumpkin whereas crude fiber, fat and ash levels can be enhanced with 14.08mGy, 10.08mGy and 6.75mGy x-ray treatment respectively.

VI. Recommendations

The overall results suggest that x-ray doses 6.75mGy, 10.08mGy and 14.08mGy can be used to increase ash, fat and crude fiber levels in fluted pumpkin respectively. Furthermore, mutant generation lines should be advanced to M_3 and above if effective and more reliable selection is desired. The chlorophyll content of the leaves of the fluted pumpkin should be studied as this contributes to the yield and nutrient level of the plant. The effect of consuming this genetically modified plant material by man should also be studied, though it is a research material and not meant for consumption at this stage. Plant breeders can utilized the information generated from this study as a guide in mutation breeding of fluted pumpkin.

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